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Dynamic reorganization of photosynthetic supercomplexes during environmental acclimation of photosynthesis

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Plants and algae have acquired the ability to acclimate to ever-changing environments in order to survive. During photosynthesis, light energy is converted by several membrane protein supercomplexes into electrochemical energy, which is eventually used to assimilate CO_2. The efficiency of photosynthesis is modulated by many environmental factors such as quality and quantity of light, temperature, drought, and CO_2 concentration, among others. Accumulating evidence indicates that photosynthetic supercomplexes undergo supramolecular reorganization within a short time frame during acclimation to an environmental change. This reorganization includes state transitions that balance the excitation of photosystem I and II by shuttling peripheral antenna proteins between the two, thermal energy dissipation that occurs at energy-quenching sites within the light-harvesting antenna generated for negative feedback when excess light is absorbed, and cyclic electron flow that is facilitated between photosystem I and the cytochrome *bf* complex when cells demand more ATP and/or need to activate energy dissipation. This review will highlight the recent findings regarding these environmental acclimation events in model organisms with particular attention to the unicellular green alga *C. reinhardtii* and with reference to the vascular plant *A. thaliana*, which offers a glimpse into the dynamic behavior of photosynthetic machineries in nature.

Keywords: acclimation, electron transfer, light-harvesting complex, non-photochemical quenching, phosphorylation

INTRODUCTION

Photosynthesis is the process of photochemical energy conversion that occurs via electron transport in the thylakoid membranes of chloroplasts, resulting in reduction of $NADP^+$ in the stroma and concomitant generation of proton motive force across the thylakoid membranes. The NADPH generated with the electron flow and the ATP produced utilizing the proton motive force are required for assimilation of carbon dioxide in the Calvin-Benson cycle. Photosystem I (PSI) and photosystem II (PSII) represent charge separation devices to drive electron flow. Although these two photosystems originated from a common prototype, the contemporary PSI and PSII complexes are rather specialized and have major differences in organization of the light-harvesting system with regard to the reaction center, pigment composition and geometry, electron acceptors and donors, and several other features. I will begin this review by introducing the current knowledge of the components and structures of the two photosystems in their normal state, and then discuss their reorganization during various acclimation events in the later sections.

Abbreviations: Chl, chlorophyll; CEF, cyclic electron flow; α-DM, n-dodecyl-α-D-maltoside; β-DM, n-dodecyl-β-D-maltoside; DBMIB, 2,5-dibromo-3-methyl-6-isopropylbenzoquinone; Cyt *bf*, cytochrome b_6f complex; DCMU, 3-(3,4-Dichlorophenyl)-1,1-dimethylurea; FCCP, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone; Fd, ferredoxin; FNR, ferredoxin-NADPH oxidoreductase; HL, high light; LHCI and LHCII, light-harvesting complex protein I and II; LEF, linear electron flow; LL, low light; NPQ, non-photochemical quenching; Pc, plastocyanin; PSI and PSII, photosystem I and II; Q, plastoquinone; WT, wild type.

PSII and its light-harvesting complex proteins (LHCII) constitute a large chlorophyll (Chl)-protein supercomplex comprising more than 30 subunits. Light energy captured by LHCIIs is transferred to the central dimeric core complex, where it is trapped and utilized to drive electron flow from water to plastoquinone (PQ). In green plants, LHCIIs are formed by two layers, i.e., (1) major "more abundant" trimeric LHCII proteins, and (2) minor "less abundant" monomeric LHCII proteins (Dekker and Boekema, 2005). In the vascular plant *Arabidopsis thaliana*, there are three major trimeric LHCII proteins (type I–III) with 5, 4, and 1 isoforms (Lhcb1.1–1.5; Lhcb2.1–2.4; and Lhcb3.1), respectively (Jansson, 1999), whereas in the green alga *C. reinhardtii* there are four major LHCII proteins (type I–IV) with 5, 1, 2, and 1 isoforms (LhcbM3, −4, −6, −8, and −9; LhcbM5; LhcbM2 and −7; LhcbM1), respectively (Minagawa and Takahashi, 2004). The three minor monomeric LHCII polypeptides CP29, CP26, and CP24 are encoded by the *Lhcb4*, *-5*, and *-6* genes, respectively, in *A. thaliana* (Jansson, 1999), whereas *C. reinhardtii* contains only the first two (Teramoto et al., 2001).

Single-particle image analysis of electron micrographs revealed that these LHCII proteins are bound to both sides of the central dimeric core complex, with the core and the major LHCII trimers bordered by a few minor LHCII monomers (Dekker and Boekema, 2005). When spinach (*Spinacia oleracea*) thylakoid membranes are solubilized by n-dodecyl-β-D-maltoside (β-DM) (Boekema et al., 1995, 1998; Hankamer et al., 1997; Nield et al., 2000b), one LHCII trimer is bound strongly to each

side of the core (C_2S_2 PSII-LHCII supercomplex), but when they are solubilized by n-dodecyl-α-D-maltoside (α-DM), the PSII-LHCII supercomplexes are organized as $C_2S_2M_{1-2}L_{0-1}$, or $C_2S_2M_0L_{1-2}$, wherein one to two moderately-bound LHCII trimers and/or one loosely-bound LHCII trimer, or one to two loosely-bound LHCII trimers, are associated with the C_2S_2-type supercomplex (Boekema et al., 1999). When *A. thaliana* thylakoid membranes are solubilized with α-DM and fractionated by gel filtration (Ruban et al., 2003) or on sucrose density gradients (Caffarri et al., 2009), the $C_2S_2M_2$ organization is the largest type observed. These single-particle data from isolated detergent-solubilized PSII-LHCII supercomplexes were recently confirmed in observations of the organization directly within the thylakoid membranes by means of cryoelectron tomography (Daum et al., 2010; Kouřil et al., 2011).

When the PSII-LHCII supercomplex from the green alga *C. reinhardtii* is prepared with a relatively high concentration of β-DM (50 mM; 2.6%), the C_2S_2 organization appears much as it does in vascular plants (Nield et al., 2000a). While a lack of M- and L-trimers in *C. reinhardtii* in an earlier report was tentatively ascribed to the absence of CP24 (Minagawa and Takahashi, 2004), which serves as a linker between PSII core subunits and an M-trimer in *A. thaliana* (Kovács et al., 2006; de Bianchi et al., 2008), both trimers were found in the more recent single particle analysis of the α-DM-solubilized PSII-LHCII supercomplex from *C. reinhardtii*, where three LHCII trimers were attached to each side of the core (the $C_2S_2M_2L_2$ PSII-LHCII supercomplex) (Tokutsu et al., 2012) (**Figure 1A**).

The supercomplex formed by PSI and its light-harvesting complex proteins (LHCI) is also a large Chl-protein complex comprising nearly 20 subunits. The PSI supercomplex collects light energy, converts it into electrochemical energy, and drives electron flow from plastocyanin (Pc) to ferredoxin (Fd). Whereas dimeric, trimeric, or tetrameric PSI cores have been reported in cyanobacteria (Boekema et al., 1987; Jordan et al., 2001; Watanabe et al., 2011), the eukaryotic PSI cores that harbor LHCIs are monomeric (Amunts et al., 2010). The association of LHCIs with a monomeric PSI core has been investigated using single-particle analysis, which revealed that in contrast to the case of the PSII supercomplex, LHCIs are asymmetrically bound to the PSI core in *C. reinhardtii* (Germano et al., 2002) and in spinach (Boekema et al., 2001) (**Figure 1**). The crescent-shaped "LHCI belt" was demonstrated to be associated with the side of the PsaF/J subunits in a 3.3 Å crystal structure of the PSI-LHCI supercomplex from pea (*Pisum sativum*) (Amunts et al., 2010). The other side of the core is unoccupied under normal conditions, exposing the PsaH/I/L subunits (**Figure 1D**), but could dock the mobile LHCII(s) under "State 2" conditions as described below. In vascular plants, the "LHCI belt" is formed by the four LHCI proteins in the order of Lhca1, −4, −2, and −3 (**Figure 1**). In *C. reinhardtii*, however, the "LHCI belt" is double layered and 9 LHCI proteins in total, encoded by the *Lhca1–9* genes (Minagawa, 2009), are attached to the side of the PsaJ/F subunits (Drop et al., 2011) (**Figure 1B**).

Because plants and algae typically do not have the means to escape adverse environmental conditions such as hot/cold temperatures, drought, high light (HL)/low light (LL), high/low CO_2 concentration, etc., the ability to acclimate is essential if they are to survive in their niche. Acclimation of the photosynthetic machinery is especially important for photosynthetic organisms to optimize their photosynthetic performance and to protect their photosynthetic machinery from photooxidative damage in the natural environment, where the quality and quantity of light fluctuates over time. This review presents an overview of the emerging evidence that photosynthesis is acclimated to environmental conditions via dynamic reorganization of photosystem supercomplexes and super-supercomplexes. This reorganization is observed during state transitions, alternating between light-harvesting and energy dissipating modes, and switching between types of electron flow. I focus on studies in model organisms (Gutman and Niyogi, 2004), such as the unicellular green alga *C. reinhardtii* and the vascular plant *A. thaliana*, and refer readers to other reviews for more comprehensive information about the acclimation events themselves including state transitions (Lemeille and Rochaix, 2010; Minagawa, 2011), excess energy dissipation (Horton et al., 2008; Li et al., 2009; de Bianchi et al., 2010), and photosynthetic electron flow (Kramer et al., 2004; Finazzi, 2005; Shikanai, 2007; Alric, 2010; Johnson, 2011).

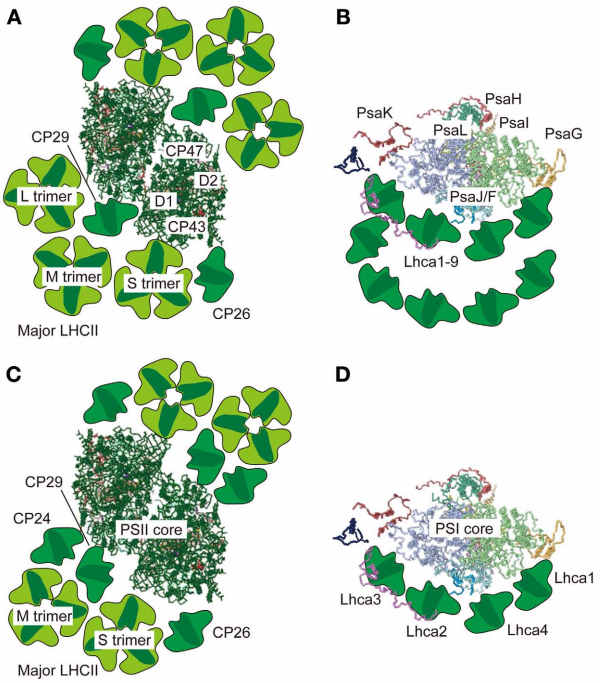

FIGURE 1 | Supramolecular organization of PSII-LHCII and PSI-LHCI supercomplexes in green algae and vascular plants. Top views of the PSII-LHCII supercomplex **(A)** and the PSI-LHCI supercomplex **(B)** from *C. reinhardtii* based on single-particle image analysis by Tokutsu et al. (2012) and Drop et al. (2011), respectively. Top views of the PSII-LHCII supercomplex from spinach **(C)** and the PSI-LHCI supercomplex from pea **(D)** based on single-particle image analysis by Dekker and Boekema (2005) and crystallography of the PSI-LHCI supercomplex (Amunts et al., 2010), respectively. All top view images are from the lumenal side. The PSII and PSI core structures were taken from the coordinates determined by crystallography in 3ARC.pdb and 2WSC.pdb, respectively.

STATE TRANSITIONS

Each of the two charge-separation devices—PSI and PSII—in the thylakoid membranes has a distinct pigment system with unique absorption characteristics. Thus, an imbalance of energy distribution between the two photosystems tends to occur in natural environments, where light quality and quantity fluctuate with time (Allen, 1992; Bellafiore et al., 2005). Since the two photosystems are connected in series under normal conditions, plants and algae constantly need to balance their excitation levels to ensure optimal efficiency of electron flow. State transitions take place under such conditions to balance the light-harvesting capacities of the two photosystems in order to minimize unequal distribution of light energy. State 1 occurs when PSI is over-excited and the mobile antennas are more associated with PSII to correct the imbalance, which can be monitored based on a higher Chl fluorescence yield at room temperature. Conversely, State 2 describes the arrangement when PSII is over-excited and the mobile antennas are accordingly more associated with PSI; State 2 is characterized by a lower Chl fluorescence yield at room temperature.

Although the core concept (Bonaventura and Myers, 1969; Murata, 1969) and the molecular mechanisms of regulation, including the involvement of Cyt *bf* (Wollman and Lemaire, 1988), binding of PQH_2 to the Qo-site of Cyt *bf* (Vener et al., 1997; Zito et al., 1999), and redox-dependent LHCII kinase (Depège et al., 2003) (**Figure 2**) have been established, investigation into the supramolecular reorganization of PSII and PSI has begun only recently, thanks to advancements in genetic and biochemical studies in two model organisms: the green alga *C. reinhardtii* and the vascular plant *A. thaliana*. In particular, many of the recent findings have been from *C. reinhardtii*. This is due, in part, to the fact that as much as 80% of the LHCII is mobile during state transitions in this green alga (Delosme et al., 1996), whereas only 20–25% of LHCII migrates in vascular plants (Allen, 1992).

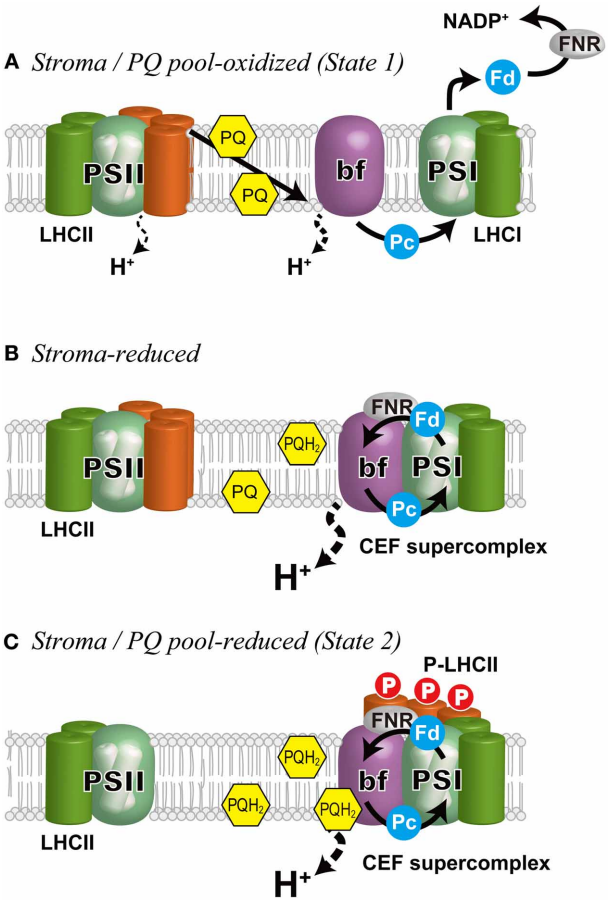

FIGURE 2 | Schematic representation of the regulation of state transitions and electron flow in *C. reinhardtii*. (A) *Stroma/PQ pool-oxidized*, when PSI is preferentially excited and the stroma and the PQ pool are oxidized. Under these conditions, LHCIIs are bound to PSII (State 1). The photosynthetic electron flow proceeds in LEF mode generating NADPH as well as a proton gradient across the thylakoid membrane that is used for ATP production. **(B)** *Stroma-reduced*, when the stroma is reduced, CEF is induced (Takahashi et al., 2013). The cytochrome b_6f complex and ferredoxin $NADP^+$ reductase (FNR) are associated with PSI to form a super-supercomplex (CEF supercomplex). **(C)** *Stroma/PQ pool-reduced*, when the PQ pool is further reduced by the elevated CEF, migration of the mobile LHCIIs (*orange*) occurs on the PsaH side of PSI that is opposite the LHCI belt to establish State 2. bf, the cytochrome b_6f complex; Fd, ferredoxin; Pc, plastocyanin.

REORGANIZATION OF PSII SUPERCOMPLEX DURING STATE TRANSITIONS

Traditionally, the PSII supercomplex has been thought to be reorganized during a State 1-to-2 transition such that some LHCIIs that form the peripheral antenna of PSII are detached upon their phosphorylation. The molecular details of this reorganization have been studied in *C. reinhardtii* (Iwai et al., 2008). Three PSII fractions corresponding to a PSII core complex, a PSII-LHCII supercomplex, and a multimer of the PSII-LHCII supercomplex were affinity-purified from a mutant expressing His-tagged CP47. Gel filtration and electron microscopy showed that the PSII-LHCII supercomplex is predominant in State 1, whereas the core complex is predominant in State 2, indicating LHCIIs are dissociated from PSII upon a State 1-to-2 transition. Moreover, in State 2, while most of the free LHCIIs are phosphorylated, most of those bound to the PSII-LHCII supercomplex are unphosphorylated, except for LHCII type I, which was found to be strongly phosphorylated in the supercomplex. The PSII subunits including the CP43 and D2 proteins in the core complex are mostly phosphorylated. Based on these findings, Iwai et al. (2008) hypothesized that (1) unphosphorylated LHCIIs stabilize the PSII complex (State 1); (2) the phosphorylation of LHCII type I in the major LHCII trimer triggers the monomerization of the supercomplexes; and (3) the phosphorylation of CP26 and CP29, as well as the PSII core subunits D2 and CP43, induces the detachment of the LHCIIs from PSII (Iwai et al., 2008). Although neither CP26 nor CP29 is phosphorylated while associated with the PSII-LHCII supercomplex, they are both phosphorylated when dissociated from PSII, suggesting that their dissociation is possibly caused by their phosphorylation. The minor monomeric LHCIIs border the major LHCII trimers and the PSII core (Harrer et al., 1998; Yakushevska et al., 2003) as shown in **Figure 1**, and the hyperphosphorylated residues in CP29 are indeed found at

the interface of the PSII core and the peripheral antenna proteins (Turkina et al., 2006). Therefore, the phosphorylation of CP26 and CP29 likely triggers undocking of the entire peripheral antenna during the State 2 transition. It should be noted that the minor monomeric LHCIIs are also shuttled to PSI, acting as a linker between PSI and major trimeric LHCII during a transition to State 2 in *C. reinhardtii* (Takahashi et al., 2006; Tokutsu et al., 2009), although this is not the case in vascular plants (Galka et al., 2012).

Recently, the remodeling of PSII supercomplex during state transitions was also studied in *A. thaliana* (Dietzel et al., 2011). Although the dissociation of LHCIIs from PSII-LHCII supercomplex correlates with the time range of the transition from State 1 to State 2, the remodeling process of the PSII-LHCII megacomplex was unaffected in the *A. thaliana stn7* mutant, which is deficient in the kinase responsible for LHCII phosphorylation (Bellafiore et al., 2005). The authors therefore concluded that PSII-LHCII megacomplex remodeling precedes the migration of LHCII from PSII to PSI, but is independent of LHCII phosphorylation and dependent on phosphorylation of a PSII core subunit CP43, which, as they found, is not dependent on the STN8 kinase previously reported to phosphorylate the PSII core subunits (Bonardi et al., 2005). Although the results from *C. reinhardtii* and *A. thaliana* show differences at some points, prompting the two research groups to propose similar but different models for state transitions, and these differences could potentially reflect differences between green algae and vascular plants, both studies indicate that phosphorylation of a PSII subunit CP43 is prerequisite for remodeling of PSII-LHCII supercomplex that precedes migration of LHCII from PSII to PSI during a state transition.

REORGANIZATION OF PSI SUPERCOMPLEX DURING STATE TRANSITIONS

Scheller and his colleagues studied the association of the mobile LHCII with PSI via cross-linking and antisense approaches. *A. thaliana* plants without PsaH and PsaL (Lunde et al., 2000), as well as those without PsaO (Jensen et al., 2004), were highly deficient in state transitions. Since these small PSI subunits are located on a vacant side of the PSI core, opposite from the LHCI belt (Amunts et al., 2010) (**Figure 1**), these PSI subunits were hypothesized to constitute a specific binding site for the mobile LHCII. The association of LHCII with PSI was first biochemically demonstrated in the *A. thaliana psae1-1* mutant: a fraction of LHCII was associated with PSI when the mutant plants were exposed to LL conditions (State 2-favoring), giving rise to a high-molecular-mass protein-pigment complex (Pesaresi et al., 2002). This large complex, however, seemed to be an aggregated product, because the mutant did not show state transitions, probably due to its low level of PsaH. The next attempt to observe LHCII with PSI was via crosslinking of the mobile LHCII proteins with the PSI-LHCI supercomplex in *A. thaliana* (Zhang and Scheller, 2004). More of the major LHCII proteins, including Lhcb1 and -2, were crosslinked to the PsaH, PsaI, and PsaL subunits in State 2 than in State 1. Further information was provided in a study on *C. reinhardtii*, wherein the PSI-LHCI-LHCII supercomplex isolated from State 2 cells contained two minor monomeric LHCII proteins, CP26 and CP29, and one major trimeric LHCII protein, LhcbM5, suggesting a pivotal role for the minor monomeric LHCII in state transitions in green algae (Takahashi et al., 2006).

The significance of the minor LHCII in state transitions in *C. reinhardtii* was supported by an RNA interference (RNAi) study in which the levels of the two minor LHCII proteins, CP29 and CP26, were individually reduced (Tokutsu et al., 2009). Both the CP29 and CP26 RNAi mutants underwent reductions in the PSII antenna size during a State 1-to-2 transition, as reflected by non-photochemical quenching (NPQ) of fluorescence, low temperature fluorescence spectra, and functional absorption cross section data. However, the LHCIIs undocked from PSII did not re-associate with PSI in the CP29-RNAi mutant, as evidenced by the fact that the antenna size of PSI was not complementarily increased. By contrast, the mobile LHCIIs in the CP26-RNAi mutant did re-associate with PSI, such that a PSI-LHCI-LHCII supercomplex could be visualized on a sucrose density gradient (Tokutsu et al., 2009). These results thus clarify that CP29, and not CP26, is crucial when mobile LHCIIs re-associate with PSI under State 2 conditions in *C. reinhardtii*.

In *A. thaliana*, the identities of LHCII trimers and specific LHCII polypeptides involved in state transitions have been controversial. At one time it was thought that the M-trimer, where Lhcb3 is almost exclusively found (Hankamer et al., 1997), was not involved in state transitions because Lhcb3 is absent from stroma lamellae under State 2 conditions (Bassi et al., 1988). However, a later report indicated that although PSII performance is not altered in a knockout *A. thaliana* mutant lacking Lhcb3, in which Lhcb1 and/or Lhcb2 replace Lhcb3 in the M trimer, the rate of transition from State 1 to State 2 is increased, suggesting that the main function of Lhcb3, and thus the M-trimer, is to modulate the rate of state transitions (Damkjaer et al., 2009). Recently, Galka et al. (2012) succeeded in purifying from *A. thaliana* and maize the most complete PSI-LHCI-LHCII supercomplex reported so far, finding that it contained a LHCII trimer with Lhcb1 and 2, but not Lhcb3. Based on the relative accumulation of the Lhcb1-2 isoforms in the PSI-LHCI-LHCII trimer complex, they concluded the LHCII is neither S- nor M-trimer and tentatively speculated that it could be an L-trimer, which is loosely bound to PSII under State 1 conditions. The fluorescence analyses indicated that excitation energy migration from mobile LHCII to PSI was rapid and efficient, and the quantum yield of photochemistry in the PSI-LHCI-LHCII supercomplex was unaffected with respect to PSI. These facts let them to suggest that rather than thinking of the mobile pool of LHCII as a part of PSII that detaches under State 2 conditions, it would be more accurate to consider it to be PSI's own antenna system that can migrate to PSII under State 1 conditions. The 2D structure of such a PSI-LHCI-LHCII supercomplex in *A. thaliana* was visualized by single particle analysis of electron micrographs (Galka et al., 2012), where a LHCII trimer was found to be bound near the PsaH/I/L site. In contrast to this observation in a vascular plant, Barber and colleagues located earlier a smaller density near PsaH, which they assigned to CP29 in *C. reinhardtii* (Kargul et al., 2005).

THERMAL DISSIPATION OF EXCESS ABSORBED ENERGY

In nature, unexpected changes in light intensity can lead to overexcitation of the photosystems, resulting in the accumulation of harmful reactive oxygen species (Li et al., 2009). Plants and algae have developed protective NPQ mechanisms that alleviate such photooxidative stress. Among these mechanisms, qE quenching—downregulation of the light-harvesting capacity of PSII—thermally dissipates excess light energy captured by PSII as a negative feed-back mechanism for the elevated electron flow. Thanks to the genetic evidence provided first by *C. reinhardtii* and then by *A. thaliana* mutants with modified NPQ capacity (Niyogi et al., 1997a, 1998), great progress has been made in elucidating the site and the mechanism of qE [see (Horton et al., 2008; Li et al., 2009; de Bianchi et al., 2010) for reviews]. In vascular plants, qE is induced upon lumenal acidification, which activates the xanthophyll cycle and a qE effector PsbS (Horton et al., 1996; Müller et al., 2001). The xanthophyll cycle, deepoxidation of violaxanthin to antheraxanthin, and then further to zeaxanthin, is catalyzed by violaxanthin deepoxidase, which is activated by low pH (Demmig-Adams and Adams, 1992). For the activation of PsbS, low pH is sensed by two glutamic acid residues on PsbS (Li et al., 2000, 2004). The elevated zeaxanthin content (Johnson et al., 2011) and protonation of PsbS (Kereiche et al., 2010) decrease the formation of ordered semi-crystalline arrays of PSII supercomplexes, leading to an increase in the fluidity of the thylakoid membranes (Goral et al., 2012). This membrane "phase transition" facilitates dissociation of several LHCII proteins forming the outer layer of the PSII-LHCII supercomplex (Betterle et al., 2009) and aggregation of the dissociated LHCII (Kiss et al., 2008; Johnson et al., 2011), which probably allows for the conformational change within the major LHCII (Ruban et al., 2007) and/or the minor LHCII (Ahn et al., 2008) to generate energy-quenching site(s).

Although both zeaxanthin and PsbS are thought to have crucial roles in qE quenching in vascular plants, the green alga *C. reinhardtii* does not express the PsbS protein (Finazzi et al., 2006; Bonente et al., 2008), even though the *PsbS* gene is present (Anwaruzzaman et al., 2004), and a mutant deficient in violaxanthin de-epoxidase activity still shows qE quenching (Niyogi et al., 1997a,b). While PsbS is present even in LL-grown plants (Demmig-Adams et al., 2006), and therefore provides constitutive photoprotection, qE in *C. reinhardtii* is not activated immediately upon exposure to HL. The activation of qE in *C. reinhardtii* requires prolonged exposure to HL (Niyogi et al., 1997a) or low CO_2 conditions (Förster et al., 2001), suggesting that algae have a distinct mechanism for qE induction and activation.

Niyogi and colleagues reported that the *C. reinhardtii npq4* mutant, which is deficient in an ancient LHC protein LHCSR3, induces little qE quenching (Peers et al., 2009). The genes for LHCSR3 (*Lhcsr3.1* and *Lhcsr3.2*), formerly known as LI818 (Gagne and Guertin, 1992), encode a 25-26 –kDa integral membrane protein whose expression is induced under HL (Richard et al., 2000), low CO_2 (Miura et al., 2004), or low iron (Naumann et al., 2007) conditions. While PsbS cannot bind pigments, LHCSR3 is capable of binding Chl *a* and *b*, as well as xanthophylls (Bonente et al., 2011) Furthermore, a recombinant LHCSR3 polypeptide reconstituted with Chl and xanthophylls is capable of dissipating excitation energy in a low pH buffer, suggesting that this protein is the primary quenching effector in *C. reinhardtii* (Bonente et al., 2011). In addition to this possible "specialized" antenna protein involved in quenching modulation, genetic analysis in *C. reinhardtii* has led to the identification of other LHCs responsible for quenching. Depletion of one of the major trimeric LHCII proteins LHCBM1 in the *npq5* mutant (Elrad et al., 2002) decreases its capacity for thermal energy dissipation. It is possible that other gene products in addition to LHCSR3 and LHCBM1 may also be involved in HL acclimation in *C. reinhardtii*.

A recent study examined where LHCSR3 is localized in the thylakoid membranes, and whether it dissipates energy captured by PSII (Tokutsu and Minagawa, 2013). By comparing the PSII-LHCII supercomplex from wild type (WT) *C. reinhardtii* cells and those of *npq4*, LHCSR3 was found to be present only in the PSII supercomplex from HL-grown WT cultures, and not in that from LL-grown WT or HL-grown *npq4*. The purified PSII-LHCII-LHCSR3 supercomplex was in a high-fluorescence state at a neutral pH (7.5), as evaluated by single-photon counting, but in an energy-dissipative state at pH 5.5, similar to the effect of lumenal acidification following HL illumination of thylakoid membranes. The switching from a light-harvesting state to an energy-dissipative state observed in the PSII-LHCII-LHCSR3 supercomplex was sensitive to dicyclohexylcarbodiimide (DCCD), a protein-modifying agent specific to protonatable amino acid residues. It is therefore likely that the association of LHCSR3 with the PSII-LHCII supercomplex is a reorganization necessary to dissipate excess absorbed energy under HL conditions in *C. reinhardtii* (**Figure 3**).

CYCLIC ELECTRON FLOW AROUND PHOTOSYSTEM I

Electrons generated in the photosystems flow into two different pathways in the thylakoid membranes—linear electron flow (LEF) from water to $NADP^+$ via PSII and PSI in series, and CEF around PSI (Arnon et al., 1958) (**Figure 4**). Although it is crucial to achieve the proper balance (3:2) of ATP and NADPH in the stroma in order to assimilate CO_2in the Calvin-Benson cycle, this balance cannot be achieved by LEF alone (Allen, 2003). As illustrated in **Figure 4**, 4 protons are released to the lumen and 2 molecules of reduced PQ (PQH_2) are released to the intersystem pool from PSII, where 4 turnovers of PSII occur upon capturing 5 photons, assuming the quantum yield of the PSII photochemistry is ∼0.8 (Björkman and Demmig, 1987). At the Qo site of Cyt *bf*, 2 molecules of PQH_2 release 4 protons to the lumen and transfer 2 electrons toward Pc. This yield of protons and electrons are doubled by means of Q cycle mechanism (Sacksteder et al., 2000). After all, 12 protons are released to the lumen and 4 Pc and then 4 $NADP^+$ are reduced by LEF. Protons released to the lumen are used to rotate the proton turbine in the CFo subcomplex of chloroplast ATP synthase, where the CF_1 subcomplex phosphorylates 3 ADP molecules per 1 rotation of the CFo. Because the number of proton-binding c-subunit in the CFo is 14 (Seelert et al., 2000), 12 protons generated by LEF can only synthesize 2.6 molecules of ATP. In order to synthesize 3 molecules of ATP, 1 electron from PSI thus needs to be reused by Cyt *bf*, namely CEF, to achieve an ATP: NADPH ratio of (3:2). CEF is thus essential for photosynthetic organisms to run productive electron transport (Munekage et al., 2004). The scheme described above manifests the quantum yield of PSII, where 5

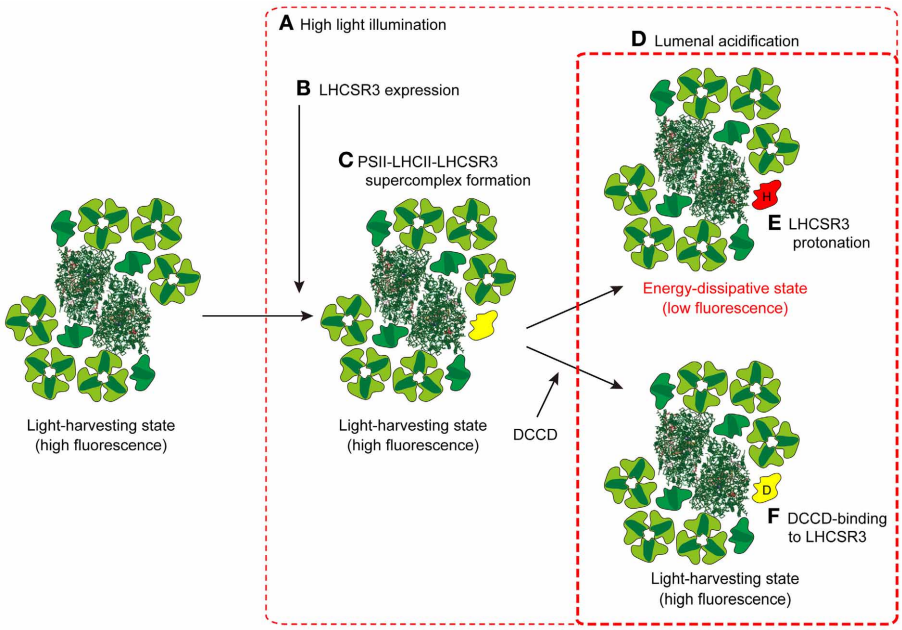

FIGURE 3 | A model for the induction of qE in *C. reinhardtii*. When *C. reinhardtii* cells are exposed to HL for several hours **(A)**, the expression of LHCSR3 is induced **(B)**. LHCSR3 (*yellow*) is then associated with the PSII-LHCII supercomplex to form the PSII-LHCII-LHCSR3 supercomplex **(C)**. Though the PSII-LHCII-LHCSR3 supercomplex is still in a light-harvesting state under dark or LL conditions, it becomes energy-dissipative upon protonation of LHCSR3 (*red*; "H" denotes protonation) **(E)** in the acidified thylakoid lumen under HL conditions **(D)**. DCCD-binding to LHCSR3 (*yellow*; "D" denotes DCCD) inhibits the conversion of PSII-LHCII-LHCSR3 supercomplex from the light-harvesting state to the energy-dissipative state **(F)**. The crystal coordinates were obtained from the Protein Data Bank: PSII core, 3ARC; LHCII, 2NHW; and PSI-LHCI supercomplex, 2WSC.

photons are required for 4 turnovers, is lower than that of PSI, where 5 photons are required for 5 turnovers (**Figure 4**). When light energy is equally distributed between the two photosystems, the PSII quantum yield is 20% lower than PSI because 20% of electrons on PSI are circulating in the CEF pathway. Since ATP and NADPH could be consumed in various cellular reactions, each demand fluctuates from time to time so that photosynthetic organisms need to constantly adjust the relative ratio of the two electron flow modes. Furthermore, CEF has another crucial role, namely building high proton motive force across the thylakoid membrane, which is especially important under adverse environmental conditions to induce NPQ mechanism as described in the previous section. Chloroplasts thus respond to the energy status and stress conditions of the cell by modulating the rate of CEF.

In *C. reinhardtii*, it has been suggested that the modulation of CEF is linked to state transitions (Finazzi et al., 2002). When the light-induced reduction of Cyt *bf* was examined in State 1- and State 2-adapted cells, differential sensitivity to the PSII inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was observed. DCMU blocked the reduction of Cyt *bf* in State 1, but not in State 2, whereas sensitivity to the inhibitor of Cyt *bf* 2,5-dibromo-3-methyl-6-isopropylbenzoquinone (DBMIB) was identical in State 1 and State 2, suggesting that PSII-independent but CEF-dependent Cyt *bf* reduction occurred only in State 2 (Finazzi et al., 1999). Furthermore, in the *stt7* mutant, which is locked in State 1 because of the lack of kinase for LHCII phosphorylation (Depège et al., 2003), electron flow remained sensitive to DCMU even under conditions in which the PQ pool was reduced (Finazzi et al., 2002). Thus, it seems likely that upon preferential excitation of PSII (State 2), CEF becomes predominant, whereas LEF predominates upon preferential excitation of PSI (State 1).

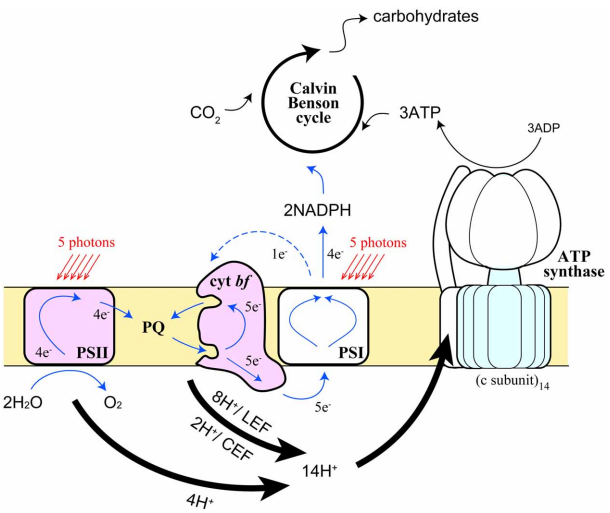

FIGURE 4 | Schematic representation of the movement of protons and electrons by the photosynthetic electron transport chain in the chloroplast. Pathways and stoichiometry of light-driven electron transport, proton translocation, and ATP synthesis on the thylakoid membranes are shown. *Thick arrows* represent the pathways of protons, *blue solid* and *blue dashed arrows* represent LEF and CEF, respectively.

Recently, the machinery for CEF was biochemically identified by utilizing the conditions leading to State 2 (Iwai et al., 2010). Thylakoid membranes from WT *C. reinhardtii* cells were first treated with carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), which depletes intracellular ATP pools by impairing respiration and stimulates glycolysis according to the Pasteur effect (Bulté et al., 1990), causing the stroma, and then the PQ pool, to be reduced. Among the solubilized membrane supercomplexes, a super-supercomplex composed of the PSI-LHCI supercomplex with LHCIIs, Cyt *bf*, Fd-NADPH oxidoreductase (FNR), and an integral membrane protein PGR5-like 1(PGRL1) (Dalcorso et al., 2008) was found in a fraction heavier than the PSI-LHCI supercomplex (Iwai et al., 2010). Spectroscopic analyses indicated that upon illumination, reducing equivalents downstream of PSI are transferred to Cyt *bf*, while the oxidized PSI is re-reduced by reducing equivalents from Cyt *bf*, suggesting that this supercomplex is engaged in CEF (Iwai et al., 2010) (**Figure 3**). The redox carriers (e.g., PQ, Cyt bf, Pc, PSI, Fd, and FNR) shared in both CEF and LEF are potentially in competition with one another. Furthermore, the redox poise of the CEF components could be disturbed if reduced LEF components coexist. Therefore, formation of a PSI-LHCI-LHCII-FNR-Cyt *bf*-PGRL1 super-supercomplex would be advantageous because it could compartmentalize CEF by localizing the mobile electron carriers (PQ, Fd, and Pc), which would allow for the production of a functional pool of CEF components to sustain the increased CEF activity.

Further reports have provided more information about CEF in *C. reinhardtii*. Mutants in which three thylakoid membrane proteins including PgrL1, CAS (Ca^{2+}-sensor protein), and ANR1 (anaerobic response protein 1) were independently knocked down were found to have decreased CEF activity under anaerobic conditions (Terashima et al., 2012). A knock-out mutant of PgrL1 was also shown to have decreased CEF activity (Tolleter et al., 2011). Because these three proteins were detected in the CEF supercomplex, they are likely to be additional components of the CEF supercomplex under anaerobic conditions (Terashima et al., 2012). The capacity for state transitions, however, is unaffected in these mutants, so state transitions are probably not causally related to LEF/CEF switching, but rather coincidental (Terashima et al., 2012). This was supported by examining CEF activity in the State 2-locked mutant of *C. reinhardtii* (Takahashi et al., 2013). Although lateral migration of mobile LHCIIs occurred in the *ptox2* mutant, which was State 2-locked because of a lack of plastid terminal oxidase 2 (Houille-Vernes et al., 2011), CEF was negligible, much like the WT in State 1. Furthermore, WT and *ptox2*, as well as the State 1-locked *stt7*, contained CEF supercomplexes under anaerobic conditions, but not under aerobic conditions, indicating that a reduced environment in the stroma, which was experimentally generated under anaerobic conditions, is more likely than LHCII phosphorylation to be the trigger for the LEF/CEF switch. Current understanding of LEF/CEF switching can be summarized as follows: Upon reduction of the stroma, CEF is activated and causes the PQ pool to be more reduced, which then induces a State 1-to-2 transition if Stt7 kinase is present (**Figure 2**). As discussed by Takahashi et al. (2013), the mechanisms by which reducing power promotes CEF and formation of the CEF supercomplexes remain to be unraveled at the molecular level.

Last but not least to discuss is the intriguing CEF component PgrL1. PgrL1 was first described as a thylakoid integral membrane protein, a mutant deficient in which displayed a similar phenotype as *pgr5* of *A. thaliana* (Dalcorso et al., 2008), which was originally reported as an allele affecting CEF in *A. thaliana*. From among NPQ mutant lines, Munekage et al. (2002) isolated a mutant that has restricted reduction rates for $P700^+$ and PQ pool under HL conditions. Since CEF was characterized in early studies as antimycin A-sensitive (Tagawa et al., 1963), an elusive antimycin A-binding enzyme Fd-PQ oxidoreductase (FQR) was proposed to bypasses Cyt *bf* and interface with PQ pool reduction by Fd (Bendall and Manasse, 1995). Because *pgr5* affected the antimycin A-sensitive electron transfer pathway, Pgr5 was assumed to be a part of FQR (Shikanai, 2007). Upon oxidation of its redox-active cysteine residues, PgrL1 from *A. thaliana* forms a complex with PGR5. This PgrL1-Pgr5 complex is proposed to associate with PSI and is reduced by Fd in a PGR5-dependent manner; the reduced PGRL1 may then be monomerized and migrate toward Cyt *bf* where it reduces PQ in an antimycin A-sensitive fashion (Hertle et al., 2013). Whereas the genes for both PGR5 and PGRL1 are present in the genome of *C. reinhardtii* (Merchant et al., 2007), only PGRL1 is included in the CEF supercomplex (Iwai et al., 2010). Protein expression of PGR5 in *C. reinhardtii* has not been confirmed under the conditions so far tested. This difference between a vascular plant and a green alga might reflect a difference in the primary mechanism for CEF between the two organisms.

CONCLUDING REMARKS

The availability of a phenomenal crystal structure of the PSII core complex at 1.9 Å (Umena et al., 2011) demonstrates that our journey of photosynthesis research in the static world is approaching its goal, which in turn opens the door to the world of dynamism. Now that the static world is becoming crystal clear, exploring the dynamic world represents one of the next challenges for biologists. Photosynthesis is dynamically regulated in nature, as required by environmental and developmental cues. However, such phenomena are extremely complex and their exploration has inevitably been difficult. Thanks to recent technical advances, including a large variety of mutant banks, purification methodology for large membrane supercomplexes, powerful proteomic approaches aided by genomic information, and computer-aided electron microscopy, those complex research topics are now within our reach.

The idea of acclimation via supramolecular reorganization of protein complexes is not particularly new, nor is it unique in the field of biology. However, what takes place in thylakoids is a large-scale and dynamic reorganization of the supercomplexes and because those are in most cases triggered simply by special light cues, the events in their entirety are truly amazing. We see a typical example in the recent study of HL-grown *C. reinhardtii* cells (Allorent et al., 2013). The respective roles of qE and qT in photoprotection were studied in mutants (*npq4*, *stt7*, *npq4*/*stt7*) and WT. Both state transitions and qE were induced

by HL and the double mutant exhibited increased photosensitivity with respect to the single mutants and the WT, suggesting that besides qE, state transitions also play a photoprotective role during HL acclimation. In addition, a line of evidence for "state transition-dependent migration of the qE effector LHCSR3" was presented (Allorent et al., 2013).

In this article, I have summarized the various approaches that have been utilized during the last 10 years or so. State transitions, excess energy dissipation, and CEF have been paid particular attention by researchers and numerous new findings are being reported. The green alga *C. reinhardtii* and the vascular plant *A. thaliana* are the two almost exclusively studied organisms in this new area. The accumulated findings indicate there are several differences in the results from these two species, for instance in the role of PgrL1 in CEF, the identity of the qE effector, and the significance of state transitions. Whether the differences can be explained only in evolutionary terms or they are instead merely superficial differences and represent small parts within some unified mechanisms are expected to be clarified in the near term.

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