FEBS Letters 587 (2013) 3372–3381

journal homepage: www.FEBSLetters.org

Review

Towards a critical understanding of the photosystem II repair mechanism and its regulation during stress conditions

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ARTICLE INFO

Article history:
Received 3 July 2013
Revised 10 September 2013
Accepted 11 September 2013
Available online 19 September 2013

Edited by Richard Cogdell

Keywords:
Photodamage and PSII repair cycle
STN8 kinase and PSII core proteins phosphorylation
Proteases and D1 protein degradation
Reactive oxygen species (ROS)
Ultra-violet radiation

ABSTRACT

Photosystem II (PSII) is vulnerable to high light (HL) illumination resulting in photoinhibition. In addition to photoprotection mechanisms, plants have developed an efficient PSII repair mechanism to save themselves from irreversible damage to PSII under abiotic stresses including HL illumination. The phosphorylation/dephosphorylation cycle along with subsequent degradation of photodamaged D1 protein to be replaced by the insertion of a newly synthesized copy of D1 into the PSII complex, is the core function of the PSII repair cycle. The exact mechanism of this process is still under discussion. We describe the recent progress in identifying the kinases, phosphatases and proteases, and in understanding their involvement in the maintenance of thylakoid structure and the quality control of proteins by PSII repair cycle during photoinhibition.

1. Introduction

In nature, abiotic and biotic stresses are the primary cause of crop loss worldwide. It has been reported that more than 50% reduction in the average yield of main crops is due to abiotic stresses [1]. Absorption of light energy by photosystem II (PSII) generates strong oxidants capable of splitting water molecules. Evolution of PSII, with the ability to utilize water molecules as a source of electrons, was a significant event for the development of life on earth. It also contributed to the gradual accumulation of oxygen in the atmosphere, thereby, permitting the evolution of oxidative phosphorylation. Indeed, many scientists refer to PSII as 'the engine of life on earth', and consider it as the most dynamically regulated part of the photosynthetic apparatus for light reaction.

All oxygenic photosynthetic organisms use light during photosynthesis to convert it into chemical energy. Thus, light is the sole energy source for photosynthesis, but ironically excess light also causes damage to the photosynthetic apparatus or in combination with other environmental stresses, including high temperature and UV-B radiation. Therefore, plants have developed several protective mechanisms against excess light. One of these mechanisms is non-photochemical quenching (NPQ) of chlorophyll fluorescence that can dissipate excessive light energy absorbed by chlorophyll as heat. NPQ is further subdivided into three components according to their relaxation kinetics in darkness following a period of illumination, as well as their responses to different inhibitors. The major component, qE, relaxes fast, within seconds to minutes, and is triggered by an increase in the ΔpH. Second component, qT, relaxes rather slowly and is due to the phenomenon of state transition. Third component, qI, is the slowest component and is

Abbreviations: Cyt *b6f*, cytochrome b6f complex; D1, a 32 kDa PSII reaction center protein which involves in PSII repair; HL, high light; LHCII, light harvesting complex II; NADPH, nicotinamide adenine dinucleotide phosphate; NPQ, non-photochemical quenching; OEC, oxygen evolving complex; PSI, photosystem I; PSII, photosystem II; qE, energy depending quenching; qI, photoinhibitory quenching; qT, quenching related to state transitions; RC, reaction center; ROS, reactive oxygen species; TEM, transmission electron microscope; UV-B, ultra-violet radiation B

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0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.09.015

related to photoinhibition involving irreversible photodamage to the D1 protein of photosystem PSII reaction center [2].

Under the photoinhibitory condition several highly toxic reactive oxygen species (ROS) are generated that contribute to oxidative damage and eventually lead to degradation of the D1 protein, one of the two reaction center proteins of PSII that is involved in PSII repair during photoinhibition [3,4]. ROS suppress the *de novo* synthesis of the new D1 copies, too [5–7]. In addition, proteins can be modified by a large number of reactions involving ROS, and among them carbonylation evokes a great deal of attention due to its irreversible nature [8]. To detoxify ROS, plants have a well-regulated antioxidant network that can be considered as an equally important protection mechanism as NPQ.

Regardless of the protective roles of NPQ and antioxidant systems, the PSII centers are prone to damage at all light intensities and hence, an efficient PSII repair cycle is required to regain the function of the PS II complexes [9–15]. PSII repair cycle is a complex process which includes photodamage of PSII reaction center, in particular the D1 protein and its phosphorylation, dephosphorylation and subsequent degradation by D1 specific kinase, phosphatase and proteases, respectively, and the cycle ends with reassembly of active PSII with newly-synthesized D1 protein (Fig. 1) [9,16]. Phosphorylation and dephosphorylation of PS II core proteins along with their subsequent degradation are essential components of the quality control and turnover of the PSII core subunits, in particular the D1 protein through PSII repair cycle [17–20]. Therefore, finding of specific kinases, phosphatases and proteases involved in PSII repair cycle is essential for the understanding of PSII repair mechanism. Furthermore, a specific role has been assigned to the stacking and unstacking of thylakoid membranes in the quality control of PSII during various stresses [21]. Recently, it has been shown that the ability of PSII to form semi-crystalline arrays in the grana membranes is strongly reduced in HL conditions [22].

Although great progresses have been made in recent years in elucidating the enigmatic dogma of the PSII repair mechanism, the exact mechanism of this process appears as a daunting task to be concluded in a straightforward way. Despite detailed structural information on the fully assembled PSII complex, the dynamic aspects of formation, processing, turnover and degradation of PSII are still not fully understood. Transient complexes are especially difficult to characterize due to low abundance, potential heterogeneity, and instability. Our understanding of this process has been enhanced significantly due to recent molecular genetic studies on genes encoding several key enzymes and subunits involved in PSII repair cycle. In this review, we will highlight the most recent progress made in solving the regulatory mechanism of PSII repair cycle and discuss the approaches that might lead to a compressive understanding of PSII repair cycle during photoinhibition.

2. Process of PSII repair cycle during photoinhibition

Photodamage of PSII occurs even in low light, and the extent of damage is proportional to the intensity of light [10,11,23–26].

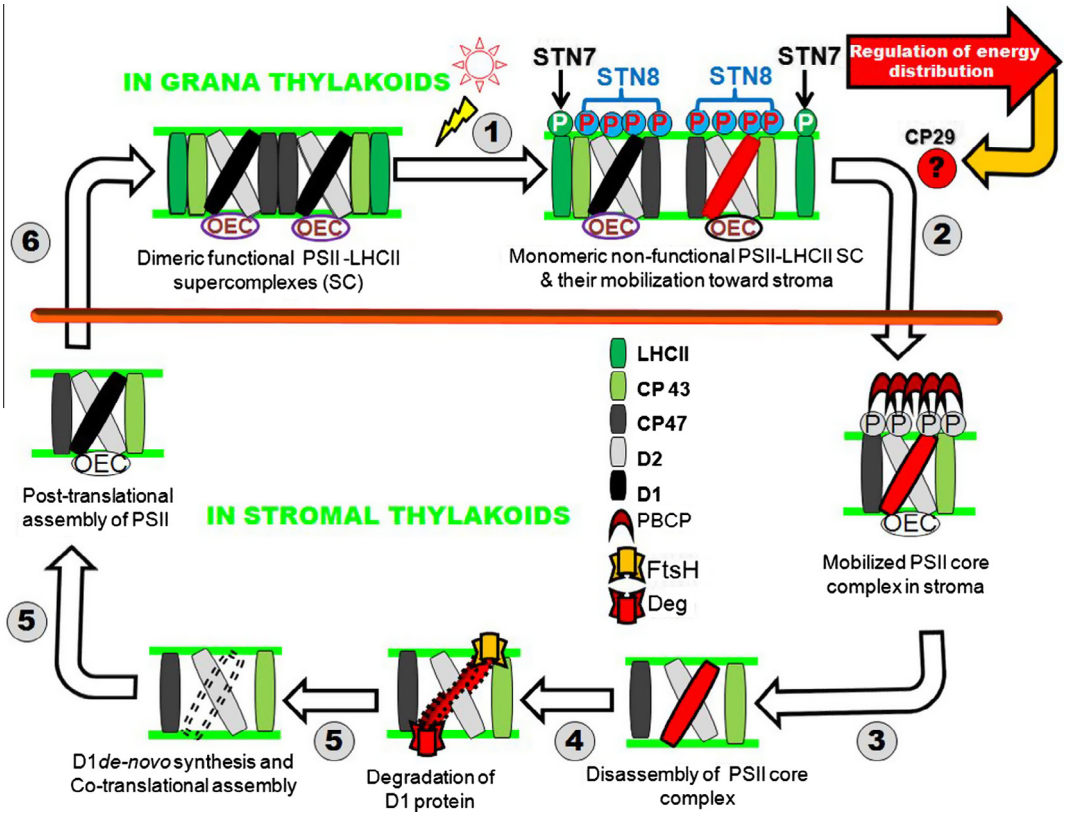

Fig. 1. Schematic representations of sequential events in PSII repair cycle during HL illumination. LHC II and PSII core proteins in dimeric functional PSII complex are phosphorylated in light by STN7 and STN8 kinases, respectively. STN7 kinase mediated LHC II proteins phosphorylation regulates energy distribution between PSI and PSII but has no significant role in PSII repair cycle (shown as red arrow). PPH1/TAP38 and PBCP phosphatase are required to dephosphorylate the LHC II and PSII core proteins, respectively. The possibility of cross-talk of STN7 with STN8 shown in Arabidopsis is indicated by a question mark and a yellow arrow toward the PSII repair cycle in which PSII core proteins phosphorylation is under the control of STN8. STN8 kinase is involved in PSII repair mechanism by phosphorylation of the PSII core proteins for proper disassembly of PSII which in turn regulates the further spatio-temporal steps of the PSII repair cycle (shown by white open arrows). Numbers 1–7 in circles briefly explain the sequential events during the PSII repairs cycle. (1) Phosphorylation of LHC II and PSII core proteins by STN7 and STN8 kinases, respectively under light. (2) Mobilization of non-functional PSII complex toward stroma region. (3) Dephosphorylation of PSII core proteins by PBCP phosphatases. (4) Proteolytic degradation of photo-damaged D1 protein by D1 specific proteases. (5) Co-translation and de novo synthesis of pre-D1 protein to be inserted into the PSII complex. (6) Post-translational modification of pre-D1 protein. (7) Reassembly of dimeric functional PSII complexes in grana.

Thus, the damaged D1 protein should be repaired at all light intensities, and the overall photosynthetic activity is reduced only when the rate of light damage exceeds the rate of repair [19,27]. Phosphorylation of PSII core proteins has been hypothesized to function as a signal for the migration of photodamaged PSII core complex from grana membranes to stroma lamellae for concerted degradation and replacement of photodamaged D1 protein. To prevent the accumulation of photodamaged PSII proteins, photosynthetic organisms have developed a multi-step process known as PSII repair cycle. As shown in Fig. 1, for the turnover of the damaged PSII core proteins, in particular the D1 protein, they must go through the following highly organized steps of PSII repair cycle: (1) reversible phosphorylation and dephosphorylation of PSII core proteins by the STN8 kinase [28,29] and PBCP phosphatase [30], respectively, (2) monomerization and disassembly of PSII complexes, (3) proteolytic degradation of damaged D1 protein, (4) de novo synthesis of D1 protein and its insertion into the partially disassembled PSII complex and (5) reassembly of the PSII complex.

2.1. Dynamics of reversible phosphorylation and dephosphorylation of PSII core proteins

Several PSII core proteins such as D1, D2, CP43, and PsbH as well as the light harvesting complex II (LHC II) proteins, in particular Lhcb1 and Lhcb2, are phosphoproteins reversibly phosphorylated in PSII [19,20]. LHC II protein phosphorylation is very often linked to the process of state transition that enables a change in the distribution of excitation energy between PSI and PSII [19,20]. Apart from state transitions, the phosphorylation of PSII core proteins, in particular, the D1 protein has been considered as one of the most important and genuine protective mechanism against the irreversible damage of PSII through the PSII repair cycle [18–20,31,32]. In the PSII repair cycle, the phosphorylation of PSII core proteins proceeds before the proteolytic degradation of damaged D1 protein, and this phosphorylation of the PSII polypeptides prevents its degradation by proteases [33].

Kinases specific to LHC II and PSII core proteins phosphorylation have been identified as STN7 and STN8, respectively [28,29], and the availability of their single and double mutants in *Arabidopsis thaliana* (hereafter Arabidopsis) has made it easier to understand the regulatory mechanism of PSII core protein phosphorylation during PSII repair cycle. Recent studies on *stn7, stn8* and *stn7x8* mutants have revealed that PSII core protein phosphorylation does play a significant role in PSII repair cycle during photoinhibition by facilitating an efficient degradation and migration of damaged PSII reaction center proteins from appressed membranes to the non-appressed membranes [19]. Further, phosphorylation of PSII core proteins by the STN8 kinase modulates macroscopic folding and rearrangement of the entire thylakoid membrane, which facilitates the lateral mobility of thylakoid membrane protein complexes that is required for PSII repair and sustained activity of PSII [20,32]. So far, the STN8 kinase have been reported to be specific for PSII core protein phosphorylation [28,29], but it possibly influences the kinetics of LHC II phosphorylation under photoinhibitory illumination as well [19]. Similarly, STN7 is involved in LHCII protein phosphorylation [28,34], but it can also contribute to the phosphorylation of the PSII core proteins [19]. The more efficient inhibition of PSII core protein phosphorylation observed in the Arabidopsis *stn7xstn8* double mutant [19,20] suggested that reversible phosphorylation of PSII proteins is required in PSII repair cycle.

However, the classical role of phosphorylation of the damaged D1 protein in PSII repair cycle has been challenged on the basis of characterization of Arabidopsis *stn8* and *stn7xstn8* double mutant [31]. The mutation of both kinase genes did not render PSII more sensitive to photoinhibition, and hence, it was suggested that the STN8-mediated PSII core protein phosphorylation is not crucial for D1 protein turnover and PSII repair. In contrast to this report, it has been shown that the mutation of *osstn8* alone is sufficient to produce all the phenotypes observed in the *stn7xstn8* double mutants of Arabidopsis, which suggests that the overlap of the function or cross-talk between STN7 and STN8 for PSII core protein phosphorylation is much less in the monocot model plant, rice, compared with the model dicot plant, Arabidopsis. Therefore, most of the results obtained with Arabidopsis *stn7xstn8* double mutants were similar to those obtained with the *osstn8* single mutant of rice [35], and all characteristic features of the *osstn8* mutant described in rice seem to be the direct consequences of the suppression of PSII core protein phosphorylation, supporting the essential role of STN8 mediated PSII core protein phosphorylation, in particular D1 protein, in PSII repair mechanism during photoinhibition.

Regulatory systems that operate via protein phosphorylation must comprise activities that turn off phosphorylation-induced signals in response to changing conditions. In photosynthetic organisms phosphorylation-triggered signals are turned off by the reversible release of the phosphate group by phosphatases, rather than by the irreversible degradation of phosphoproteins by proteases [36]. Compared with the extensive studies on the roles of kinases and proteases involved in PSII repair cycle, the roles of specific phosphatases in PSII repair cycle have yet to be explored. Earlier reports have shown that the activity of a phosphatase working on a specific thylakoid phosphoproteins is different to other phosphatases in terms of their dephosphorylation kinetics. LHC II proteins are the most rapidly dephosphorylated, followed by D1 and D2, CP43 and PsbH [37]. Recently, two protein phosphatases, phosphatase1/thylakoid-associated phosphatase38 (PPH1/TAP38) and PSII core phosphatase (PBCP) were identified to efficiently counteract the activities of the STN7 and STN8 kinase, respectively [30,38,39]. Although PPH1 is specific for dephosphorylation of STN7 mediated phosphorylation of LHCII proteins, no co-expression at transcript level of genes coding for PPH1 with that of STN7 was observed [40], suggesting that LHCII dephosphorylation is probably not the only function of PPH1. Situation is different for PBCP that dephosphorylates STN8-mediated PSII core phosphoproteins and is involved in thylakoid stacking [30]. Chloroplast calcium sensor protein CAS as well as PGRL1 were also shown to be targets of STN8 [29], but the specific phosphatases have remained elusive. There is some overlap in the function of PPH1 and PBCP because plants that overexpress PBCP are affected in state transitions and also show slightly altered LHC II phosphorylation kinetics [30]. Furthermore, the ATTED II co-expression network of PBCP comprises STN8, SIG3, and CSK in close proximity, which could suggest that PBCP not only counteracts STN8 activity but also has a role in the dephosphorylation of the plastid encoded RNA polymerase (PEP) complex and/or sigma factors. Thus, more extensive study in future is required to elucidate the mechanism of action of phosphatases in terms of when and how they are involved in dephosphorylation of photodamaged D1 protein before the degradation takes place.

It remains unclear whether LHC II protein phosphorylation is involved in PSII repair mechanism. Some recent works have indicated that phosphorylation of CP29, one of the three minor chlorophyll a/b-binding protein associated with PSII, has a role in state transitions and also in the disassembly of PSII–LHC II supercomplexes [41]. Dynamic disassembly of PSII–LHC II supercomplexes is likely to help in the lateral migration of PSII supercomplex from grana stacks to stroma lamella (Fig. 1), an essential step in PSII repair cycle [41].

2.2. Monomerization and disassembly of PSII complexes

Both the degradation of damaged PSII protein subunits and their replacement by the de novo synthesized copy take place on

stroma membrane after the migration of phosphorylated damaged monomeric PSII complex from grana to stroma thylakoids [42,43]. Therefore, it is proposed that the repair of PSII requires at least partial disassembly of the PSII complexes [19,43]. In chloroplasts, when the repair process of PSII is blocked by chloroplast protein synthesis inhibitor such as lincomycin, PSII monomer complexes disappear from the stroma thylakoid region [19]. This hypothesis is supported by a recent finding showing that the *stn7xstn8* mutant, as compared to wild-type is unable to operate PSII monomerization upon prolonged HL illumination, and thereby the mutant plants still contain photodamaged PSII core dimers even in PSII supercomplexes [19]. In this study, the distribution of PSII complexes between the monomers, dimers and supercomplexes was similar in the control condition between wild-type and *stn7x stn8* double mutants, where the PSII core protein phosphorylation is completely impaired. However, when leaves of both genotypes are treated with lincomycin prior to the exposure to HL illumination, in order to prevent the synthesis of the new copies of the D1 protein, a rapid disassembly of PSII supercomplexes is observed only in wild-type suggesting that the *stn7x stn8* double mutant is impaired in the efficient disassembly of PSII supercomplexes. The lack of PSII core protein dephosphorylation disturbs the disassembly of PSII supercomplexes under HL illumination, which is a prerequisite for efficient migration of damaged PSII complexes from grana to stroma lamella for repair through PSII repair cycle [19].

2.3. Dynamical structural changes of grana network and mobilization of damaged protein complexes during photoinhibition in thylakoid membranes

While talking of PSII repair cycle and distribution of various proteases as well as their interaction with damaged D1 protein, it becomes essential to describe the role of grana stacking and thylakoid membrane heterogeneity. Stacking and unstacking of thylakoid membranes are controlled by electrostatic interactions of the membrane surface in general [44] and thus is affected by the ionic environment around it [45,46]. Most likely, it is the hydrogen bonding and the electrostatic interactions that participate in membrane stacking. Several stress factors like strong light or heat may change these interactions, thereby, inducing unstacking of the thylakoid membranes. Under natural conditions, stacking and unstacking of thylakoid membranes can occur within minutes and are mostly reversible processes [47].

About 80% of thylakoids in higher plants are known to be stacked in darkness [48], and thylakoid stacking may play a critical role in stabilizing PSII and collecting excitation energy, while thylakoid unstacking may be essential to avoid further damage to PSII by ROS produced in PSII under strong illumination [49]. A pivotal role of stacking and unstacking of thylakoid membranes has been demonstrated in the quality control of PSII under HL illumination [22] and also during heat stress [50]. Accordingly, there are three major ways by which unstacking of thylakoid membranes can affect the turnover of the D1 protein: i) unstacking facilitates diffusion of PSII complexes from the grana to grana margin and the stroma membrane enabling efficient repair of damaged PSII complexes, ii) unstacking may dramatically enlarge the area of grana margin which increases the chances to encounter proteases to recognize and digest the damaged D1 protein, and iii) unstacking may protect PSII from damage by ROS [22]. More ROS has been found to be produced in stacked thylakoids than in unstacked ones [48,49].

As we explained above that PSII core protein phosphorylation by STN8 kinase is essential for the monomerization of PSII dimer complexes and a prerequisite for fluent mobilization of damaged PSII complexes from grana to stroma lamella region for its degradation [19]. Interestingly, an increase in grana size in *stn7xstn8* double mutant of Arabidopsis as compared to wild-type leaves was observed by transmission electron microscopy (TEM) analysis [20]. However, our recent data [35] indicate that the *osstn8* mutant phenotype in rice is even stronger than that in Arabidopsis. On the basis of data, now published both on Arabidopsis and rice, [19,20,35], we provide a new model on PSII repair cycle (Fig. 2) that makes it possible to understand the complex process of PSII repair cycle during photoinhibition. TEM analysis data of rice thylakoid network, as shown in Fig. 3, revealed that although the stacking of grana is significantly reduced in chloroplasts, the length of grana is dramatically increased in the *osstn8* mutant of rice as compared to wild-type under natural growth conditions. Under HL illumination at 2000 μmol photons m^{-2} s^{-1} for 3 h, a significant increase in grana stacking and a slight increase in the width of grana sacs occurred in wild-type chloroplasts, but not in the *osstn8* plants (Fig. 3). These striking features of the thylakoid membrane in *stn8* mutants suggest that the organization and proportion of grana and stroma lamellae in chloroplast play a significant role in PSII repair mechanism by suppressing the degradation of photodamaged D1 protein under HL illumination [19,20,35]. Recent work [21] has provided evidence that the structural heterogeneity of PSII supercomplexes, rather than the action of the PsbS protein, is responsible for the reduced ability of PSII to form semi-crystalline arrays in the grana membranes. Moreover, there is accumulating evidence suggesting that PSII photoinhibition-repair cycle requires brisk lateral diffusion of proteins between stacked grana and unstacked stroma membranes. In other words, HL treatment leads to reduced grana diameter and condensation of the grana network along with increased mobility of grana-hosted protein complexes [51,52].

2.4. Proteolytic degradation of D1 protein

As a part of the PSII repair cycle, damaged D1 protein should be rapidly degraded by chloroplast proteases [53–56]. The D1 protein is located in the center of PSII and embedded in the thylakoid membrane with five transmembrane helices with the N-terminal end exposed to the stromal side [57–59], and maturation of D1 protein requires a processing step at C-terminal end on the luminal side [60,61].

Mainly two families of proteases have been reported to be responsible for the proteolytic degradation of the D1 protein: the ATP-dependent zinc metalloprotease FtsH (filament temperature sensitive H) family [62] and the ATP-independent serine endoprotease Deg/Htr family [63]. Thus, the highly hydrophobic nature of the D1 protein demands coordinated degradation conducted by FtsH and Deg (and possibly other house-keeping proteases) at both sides of the thylakoid membrane (Fig. 1). Herein the question arises as how these proteases act on D1 protein degradation? It is noted that FtsH is a peptidase that recognizes an end of peptides and is responsible for processive hydrolysis of peptide bonds [64], and this progressive degradation depends on unfoldase activity provided by the ATPase domain. Consequently, these circumstances indicate that FtsH can degrade D1 by itself. In contrast, Deg is an endo-peptidase that cleaves intermediate peptide regions exposed to the lumenal side [65].Therefore, complete digestion of the D1 protein is unlikely to occur by Deg alone, and requires additional proteases. Based on these observations, it is clear that FtsH has predominant role in D1 protein degradation [66,67], whereas Deg protease assists effective D1 degradation by increasing the number of D1 endoproteolytic intermediates that are accessible to FtsH [68] during PSII repair cycle.

2.4.1. Degradation of photodamaged D1 protein by FtsH proteases

Much attention has been paid on the identification of proteases that are involved in the degradation of damaged D1 protein [53,69–76]. It is now clear that FtsH proteases play a more crucial role in D1 protein degradation than originally hypothesized.

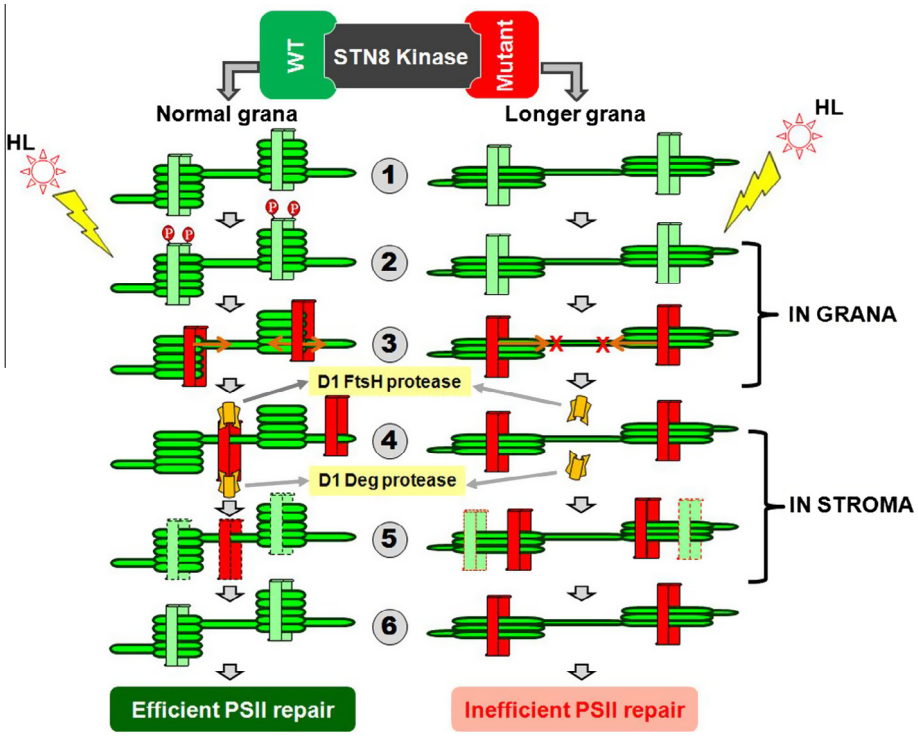

Fig. 2. Alteration of grana size and their stacking in wild-type and STN8 kinase knock-out mutant of rice (*osstn8*) and Arabidopsis (*stn8*). Schematic model representing the influence of the lack of the STN8 kinase-mediated PSII core protein phosphorylation in STN8 kinase knock-out mutants. Numbers 1–6 in the circles show the difference between the events in the mutants and wild-type during the PSII repair cycle. (1) Abnormal grana in mutants due impairment of STN8 kinase-mediated PSII core protein phosphorylation. (2) Phosphorylation of PSII core proteins do not occur in mutants even under HL illumination. (3) Core protein phosphorylation facilitates the migration of damaged PSII from grana to stroma-exposed membranes for repair. (4) D1 specific proteases, such as FtsH and Deg, cleave the damaged D1-protein and allow the further steps of the repair cycle to occur fluently (see Fig. 1). Mutants keep accumulating damaged D1 protein in the grana and the entry of newly synthesized copies of D1-ptroteins is blocked. (5) Accumulation of photodamaged D1 protein in stacking region of thylakoid membrane in mutant due to the lack of STN8 kinase-mediated PSII core protein phosphorylation. Resultant efficient PSII repair in wild-type and inefficient PS II repair in *osstn8* mutant.

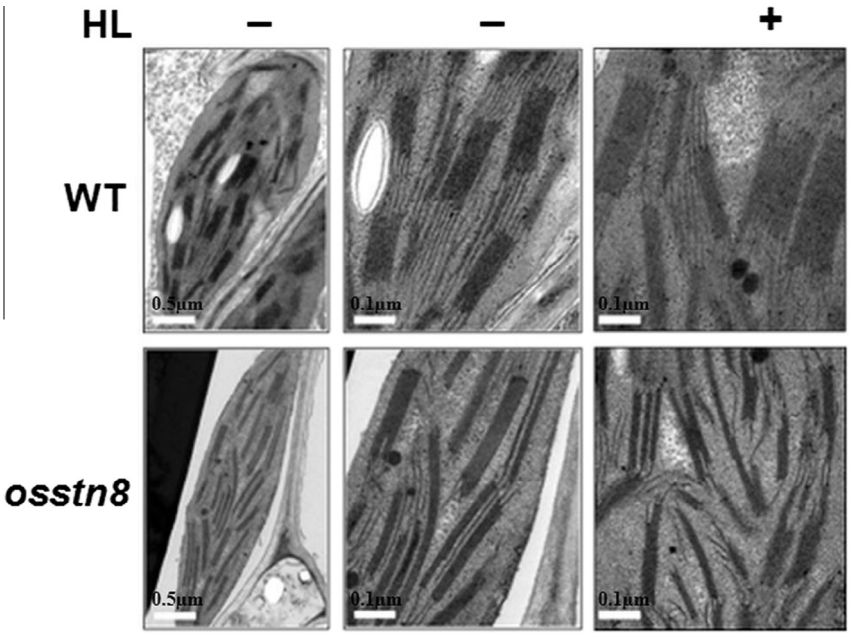

Fig. 3. Size and local distribution of grana and stroma lamella in the chloroplast of wild-type and *osstn8* mutant plants before (–) and after (+) HL illumination monitored by TEM micrographs. Dramatic alteration of grana size and their stacking in mutant were due to lack of STN8-mediated phosphorylation of PSII core proteins. Leaf fragments were illuminated at 2000 μmol photons m^{-2} s^{-1} HL intensity for 3 h. Sections shown are from wild-type and *osstn8* plants in low and high resolutions at 0.5 μm (left) and 0.1 μm (middle and right), respectively.

Mutants lacking FtsH2 in *Synechocystis* 6803 [66,67] and FtsH2 and FtsH5 in Arabidopsis [53,77] showed impaired D1 protein degradation. In Arabidopsis, 12 FtsH proteases have been identified, and nine of them are annotated as chloroplast targeted proteins [78]. In chloroplast the FtsH proteases form a hexameric heterocomplex of two pairs of redundant gene products FtsH2/8 and FtsH5/1 [62,76,78,79]. FtsH hexamers are further shown to be heteromeric, containing 'Type A' (FtsH1, FtsH5) and 'Type B' (FtsH2, FtsH8) subunits [80]. Involvement of the FtsH complex in D1 protein degradation is implicated by indirect evidence that mutants lacking FtsH2 and FtsH5 are more susceptible to photoinhibition under HL illumination [53,81]. Mutant studies showed that the presence of at least one protein from each type is essential for FtsH hexamers to accumulate and function properly [82].

Alternatively, it has also been suggested that FtsH proteases can alone degrade the D1 protein both in chloroplast of higher plants and in cyanobacteria with different mode of action. In *Synechocystis*, the proteolysis of the photodamaged D1 protein proceeds progressively from the N-terminus [83], and the degradation occurs rapidly without appearance of degradation intermediates. In contrast, D1 protein degradation in chloroplast produced two degradation fragments, a N-terminal 23 kD and a C-terminal 9 kDa fragment [9,84], and these fragments are further degraded by the subsequent proteolysis. However, direct evidence of D1 cleavage has only been reported for recombinant FtsH1, which is able to degrade D1 protein fragments, but not the full-length damaged D1 protein [85].

Similarly, among four FtsH proteases in *Synechocystis* [85], slr0228 has been reported to be responsible for degradation of light-damaged and heat-damaged D1 protein [66,67,83,87]. slr0288, one of the FtsH showing highest similarity with Arabidopsis FtsH2, plays an important role in PSII repair and degradation of the D1 protein [66,67]. However, FtsH hetero-complex remains unclear at the moment, but slr0288 protein appears to physically interact with the D1 protein [66]. Another recent report demonstrated that the D1 protein degradation is dramatically attenuated when the N-terminal 20 amino acids are removed from the D1 protein [83]. These observations suggest that the predominant role of FtsH in PSII repair is shared between *Synechocystis* and Arabidopsis.

2.4.2. Degradation of photodamaged D1 protein by Deg proteases

Unlike FtsH, Deg is not an ATP-dependent protease. In Arabidopsis, 16 Deg protease have been identified [63,86], and immunoblot analysis has revealed that four of them (namely Deg1, Deg2, Deg3 and Deg4) are located in chloroplast. In vitro studies have revealed that Deg2 cleaves the photodamaged D1 protein selectively in the stroma-exposed DE-loop [88] into N-terminal 23kD and C-terminal 10kD fragments [89], and the secondary proteolysis to remove the 23kD fragment is carried out by FtsH1 [85]. However, DEG2 knock-out mutant of Arabidopsis showed normal phenotype and has similar D1 protein turnover like wild-type under HL illumination [90] suggesting that participation of Deg2 is not essential in in vivo condition. Recent studies on *deg1* knockdown mutants of Arabidopsis have revealed that mutants are more sensitive to HL illumination. These plants accumulated higher amount of full length D1 protein and much less degraded C-terminal 16 kD and 5.2 kD products [72] as compared to wild-type plants. Similar finding is reported in *deg5* and in *deg8* single mutants as well as in the *deg5xdeg8* double mutants [54,55] suggesting no participation of these proteases in D1 protein degradation and PSII repair during photoinhibition under HL illumination.

2.5. Replacement of photodamaged D1 protein and reassembly of PSII complexes

To replace the damaged D1 protein in PSII reaction centers, incorporation of newly synthesized copy of D1 protein in PSII complex is essential. Thus, once the damaged D1 protein is degraded, a new functional copy is de novo synthesized and incorporated into the core complex using the chloroplast translation machinery. Synthesis of the precursor D1 protein (pre-D1) and replacement of the damaged D1 protein by the insertion of the newly synthesized precursor into the thylakoid membrane concomitantly with the assembly of other PSII proteins is thought to be essential to complete the PSII repair cycle. Thus, among several steps, de novo synthesis of the D1 protein is the most important step to terminate the PSII repair cycle for the reassembly of PSII supercomplex during HL illumination [42].

The newly repaired PSII core complex is reassembled with the structural and peripheral antenna complexes in the grana partition of the thylakoid membrane. Hypothetically the fully reassembled complex is now active and ready to function again. However, the recent advances in identifying the various proteins involved in PSII assembly and PSII repair are still under investigation. Psb27 has been shown to be an 11 kDa cyanobacterial lipoprotein and has been shown to have a role in biogenesis of the water-splitting site that is most critical for the function of PSII and especially for the repair of damaged PS II complexes. It is probably a part of a preassembled PSII supercomplex that represents a distinct intermediate in the repair cycle of PSII [91,92].

3. Influence of high temperature and UV-B radiations on PSII repair

Over-excitation by surfeit light can produce harmful reactive oxygen intermediates detrimental to pigments, proteins and lipids. Several protective mechanisms are stimulated when light absorption exceeds its utilization in photosynthesis. As shown in Fig. 4 the excess excitation energy is safely dissipated as heat by NPQ to protect plant not only from photodamage but also from higher amount of ROS during over-excitation of chlorophyll molecules. The major component of NPQ, referred to as energy-dependent quenching qE, is triggered by the pH gradient across the thylakoid membrane that results from the photosynthetic light reactions. qE is characterized by a decrease in chlorophyll fluorescence quantum yield. It is still a matter of debate how the major components responsible for qE like LHCII, PsbS, and zeaxanthin work together to switch reversibly from an energy transmitting state to a quenched state.

In nature, HL intensities are generally associated with high temperature as well as high UV-B radiations, reaching the plant. Thus, both the high temperature and UV-B radiations can likewise influence the PSII repair cycle. Heat stress is usually accompanied by HL, and therefore, photoinactivation of PSII is very likely to occur at high temperatures. In general, such environmental stresses are mostly responsible either for the inhibition of the capacity of photosynthetic CO_2 metabolism, and the impairment of the electron transport or the change in the status of excitation energy (Fig. 4). Heat stress is a serious threat to plants, which can lead to a drastic reduction in crop yield [93]. Exposure of plants to elevated temperatures results in an inactivation of Rubisco activase [94] and the oxygen-evolving complex (OEC) of PSII, including the removal of the extrinsic proteins as well as the release of calcium and manganese ions from their binding sites. Heat stress can also damage the D1 and D2 proteins [95]. Lower and higher temperatures than the normal physiological one modify the fluidity of the thylakoid membrane with direct effects on the PSII repair cycle [96,97].

Photoinhibition and "heat inhibition" of PSII share certain similarities in cleavage of the damaged D1 protein. However they differ in that photoinhibition does not involve the release of PsbO, P

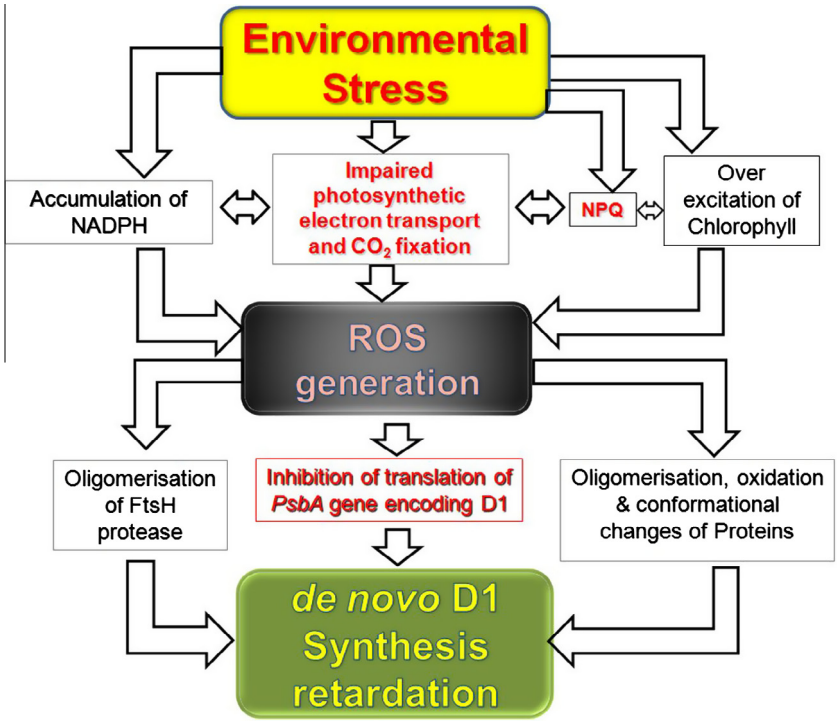

Fig. 4. Environmental stresses and endogenous factors that affect the PSII repair cycle.

and Q proteins. These extrinsic proteins are more easily removed under heat stress and as a result the damaged D1 protein tends to form aggregates which resist proteolysis by specific proteases [50].

Inactivation of PSII by excess light and damage to the D1 protein are most probably induced by singlet oxygen [98]. Ascorbate has been suggested to play a protective role against photoinhibition in heat-stressed leaves [99]. Ascorbate is present in the lumen probably at millimolar concentration [100] serving as a relatively rapid ($t_{1/2}$ approximately 25 ms) electron donor to PSII in the absence of active OEC [101] and thus might be capable of protecting PSII by supplying electrons to the reaction center.

Tang et al. [102] suggested that the aggregation of LHCII represents a protective mechanism to help the dissipation of excess excitation energy due to significant inhibition of CO_2 fixation under heat stress. Investigations on the combined action of moderate light intensity and heat stress suggest that moderately high temperatures do not cause serious PSII damage but inhibit the repair of PSII. The attack of ROS during moderate heat stress principally affects the repair system of PSII, but not directly the PSII reaction center (RC). Heat stress additionally induces cleavage and aggregation of RC proteins; the mechanisms of such processes are yet unclear. On the other hand, membrane linked sensors seem to trigger the accumulation of compatible solutes like glycine betaine in the neighborhood of PSII membranes. They also induce the expression of stress proteins that alleviate the ROS-mediated inhibition of repair of the stress damaged photosynthetic machinery and are required for the acclimation process [103].

Photoinhibition is induced by HL intensity which also contains significant amounts of UV-B radiation. UV-B radiation is absorbed by the majority of essential biological compounds: nucleic acids, proteins, pigments and lipids. High intensity UV-B radiation damages almost all components of the photosynthetic apparatus [104]. Effects of UV-B radiation include: destruction of chloroplast ultrastructure, damage to D1/D2 proteins, loss of the photosynthetic pigments [105], damage of the Rubisco enzyme [106], and a general decrease of mRNA transcripts for photosynthetic complexes [107]. UV-B radiation damages the D1 and D2 proteins almost to the same extent and the repair process includes de novo synthesis of both subunits [108]. Furthermore, it has been demonstrated that when the *slr2100* gene is inactivated in Synechocyctis 6803, the repair of UV-B damaged PSII is retarded. In the *Slr2100* mutant the level of cGMP is unregulated, which improves the tolerance of PSII apparatus to UV-B stress [109]. The participation of the FtsH/Slr0228 protease has also been shown in the repair of UV-B damaged PSII reaction center [110]. The FtsH protease is involved in the in vivo proteolytic removal of both D1 and D2 proteins of the PSII complex. In contrast, Deg proteases do not seem to have a role in PSII repair following UV-B induced damage either in D1 or D2 proteins proteolysis.

4. Role of ROS in PSII repair

Under many biotic and abiotic stress conditions, an imbalance between ROS generation and scavenging occurs, and the accumulating ROS cause damage to cells near their generation sites [111]. Even though ROS are scavenged by diverse enzymatic and non-enzymatic antioxidative defense substances [112,113], the levels of ROS may rise rapidly following environmental changes [114]. The main source of ROS in chloroplasts is the electron transport chain and the generation site for each ROS differs depending on the stress applied [112,115,116].

In the photosynthetic machinery, the conversion of excitation energy absorbed by chlorophylls into the energy of separated charges and subsequent water–plastoquinone oxido-reductase activity are inadvertently coupled with the formation of ROS. Singlet oxygen is generated by the excitation energy transfer from triplet chlorophyll formed by the intersystem crossing from singlet chlorophyll and the charge recombination of separated charges in the PSII antenna complex and reaction center of PSII, respectively. Apart from energy transfer, the electron transport, associated with the reduction of plastoquinone and the oxidation of water, is linked to the formation of ROS including superoxide anion radical and hydrogen peroxide. PSII evolved a highly efficient antioxidant

defense system to scavenge ROS and protect PSII from oxidative damage. Both the check and balance of the formation and scavenging of ROS are controlled by the energy level and the redox potential of the excitation energy transfer and the electron transport carrier, respectively [117]. Because the D1 protein is the main target of photoinhibition in PSII RC, ROS can induce the specific cleavage of the D1 protein in vitro [4,118]. Based on in vivo studies of cyanobacteria, it has also been suggested that ROS act primarily by inhibiting the synthesis of the D1protein but not by damaging PSII directly [119].

When photosynthetic tissues are illuminated in excess of the energy utilization potential of carbon reduction, there is a marked decrease in photosynthetic capacity, and this over-excitation of the photosynthetic apparatus may result in damage to PSII reaction centers [120,12]. Decline in the CO_2 fixation leads to accumulation of NADPH, which in turn renders the system to generate and accumulate higher amount of ROS in thylakoids (Fig. 4). On the other hand, environmental stresses alter the photosynthetic parameters such as NPQ which is essential for the dissipation of excess excitation energy, and Cyt *b6f* which alters the electron transport rate (Fig. 4). As a consequence of alteration in excitation energy and impairment in electron transport rate under stressful environment, the level of ROS in chloroplast increases as shown in Fig. 4. However, several earlier studies have suggested that environmental stresses accelerate photodamage to PSII [121], but recent findings demonstrated that PSII repair process is more sensitive to environmental stress than the damage of PSII, due to inhibitory action of ROS in D1 protein synthesis [5–7].

In addition to other environmental stresses affecting the repair cycle of PSII, it is evident that PSII photoinhibition as such is enhanced by abiotic stresses (Fig. 4). Here, based on the mutation of *OsSTN8* gene in rice which causes impairment in PSII core protein phosphorylation and renders *osstn8* mutants to photooxidative damage, a new model of the PSII photoinhibition-repair cycle is proposed (Fig. 2). Accumulation of damaged D1 protein is possibly due to impaired disassembly of PSII and/or modified grana stacking in mutant plants, as also reported by [16] and [17], respectively. Accumulation of damaged PSII complexes is likely to be the main reason for ROS generation in *osstn8* mutant. ROS generation due to photooxidative damage mainly leads to: (i) slowdown of D1 protein degradation, and (ii) inhibition of D1 protein de novo synthesis, during photoinhibition.

5. Conclusions and future perspectives

This review updates the recent progress in elucidating how thylakoid protein phosphorylation has evolved as a genius way to regulate the repair of PSII during HL illumination and other abiotic stresses. Further studies are needed to characterize how STN7 and STN8 interact with each other at the substrate level and whether there are species-specific differences in the cross-talk of the STN7 and STN8 kinases during photoinhibition. Thus, extensive research is required to explain the regulation and target protein cross-talk spectrum of the kinases in hierarchical order. It has been reported that phosphorylation of CP29 occurs only in monocot plants, but not in dicot plants under abiotic stresses, such as cold stress and HL illumination [41]. In this regard, the regulatory role of CP29 phosphorylation can be monitored by making use of *stn7* and *stn8* mutations in using monocot and dicot model plants, rice and Arabidopsis, respectively. This would open new windows for understanding the relationship between CP29 and the STN7 and STN8 kinases in different environmental stresses, and their involvement in PSII repair and state transitions. Sensitive proteomics approaches of thylakoid membrane might be able to reveal novel kinases that are additionally involved in PSII core and LHCII proteins phosphorylation, since the *stn7/stn8* double mutant still has trace amounts of phosphoproteins [19,20]. Moreover, it has been suggested that D1 protein is prone to oxidative damage by ROS produced by HL illumination which also hinders the de novo synthesis of the D1 protein. However it is not yet clear how ROS affect the D1 protein degradation or FtsH oligomerisation during photoinhibition. Endopeptidic cleavage of D1 protein by Deg and its subsequent degradation by FtsH proteases in PSII repair cycle are well established, but the mechanisms and involvement of other proteases such as SppA and Clp in this pathway are largely unknown. Future studies need to be focused on membrane fluidity and dynamics which are important for migration of photosynthetic complexes and their units in thylakoid membranes during HL illumination.

Acknowledgments

This work was supported by a grant from the National Research Foundation of Korea (NRF), funded by the Korea government, Ministry of Education Science and Technology (MEST) (No. 2010-0011395, 2011-0017947, 2012-0004968).

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