Biochimica et Biophysica Acta 1837 (2014) 217–225

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Oxidative stress and photoinhibition can be separated in the cyanobacterium *Synechocystis* sp. PCC 6803

Kaisa Hakkila [a], Taras Antal [a,b], Ateeq Ur Rehman [c], Juha Kurkela [a], Hajime Wada [d], Imre Vass [c], Esa Tyystjärvi [a], Taina Tyystjärvi [a,*]

[a] Department of Biochemistry, University of Turku, FI-20014 Turku, Finland
[b] Biological Faculty, Moscow State University, Vorobyevi Gory 119992, Moscow, Russia
[c] Institute of Plant Biology, Biological Research Centre of Hungarian Academy of Sciences, P. O. Box 521, H-6701 Szeged, Hungary
[d] Department of Life Sciences, University of Tokyo, Komaba 3-8-1, Meguro-ku, Tokyo 153-8902, Japan

ARTICLE INFO

Article history:
Received 4 September 2013
Received in revised form 9 November 2013
Accepted 15 November 2013
Available online 22 November 2013

Keywords:
High light
Oxidative stress
Photoinhibition
Photosystem II
Reactive oxygen species (ROS)
Sigma factor

ABSTRACT

Roles of oxidative stress and photoinhibition in high light acclimation were studied using a regulatory mutant of the cyanobacterium *Synechocystis* sp. PCC 6803. The mutant strain ΔsigCDE contains the stress responsive SigB as the only functional group 2 σ factor. The ΔsigCDE strain grew more slowly than the control strain in methyl-viologen-induced oxidative stress. Furthermore, a fluorescence dye detecting H_2O_2, hydroxyl and peroxyl radicals and peroxynitrite, produced a stronger signal in ΔsigCDE than in the control strain, and immunological detection of carbonylated residues showed more protein oxidation in ΔsigCDE than in the control strain. These results indicate that ΔsigCDE suffers from oxidative stress in standard conditions. The oxidative stress may be explained by the findings that ΔsigCDE had a low content of glutathione and low amount of Flv3 protein functioning in the Mehler-like reaction. Although ΔsigCDE suffers from oxidative stress, up-regulation of photoprotective carotenoids and Flv4, Sll2018, Flv2 proteins protected PSII against light induced damage by quenching singlet oxygen more efficiently in ΔsigCDE than in the control strain in visible and in UV-A/B light. However, in UV-C light singlet oxygen is not produced and PSII damage occurred similarly in the ΔsigCDE and control strains. According to our results, resistance against the light-induced damage of PSII alone does not lead to high light tolerance of the cells, but in addition efficient protection against oxidative stress would be required.

1. Introduction

Cyanobacteria require light for photosynthesis and growth. In natural conditions, light intensity and light quality vary, and multiple acclimation responses protect cells against photodamage [1]. Imbalance in functions of photosystems I and II (PSI and PSII) and carbon fixation may lead to enhanced production of reactive oxygen species (ROS) and up-regulation of ROS protective mechanisms in cyanobacteria [2]. In addition, light specifically damages PSII (for recent reviews, see [3,4]).

ROS in cyanobacteria include singlet oxygen ($^1O_2$), superoxide (O_2^-) and hydrogen peroxide (H_2O_2). Both respiration and photosynthesis are possible sources of ROS,and in cyanobacteria especially the role of photosynthetic electron transfer chain in ROS production has been studied [2]. $^1O_2$ is mainly produced in photosystem II (PSII) when a triplet chlorophyll (Chl) reacts with O_2. Due to its short lifetime, $^1O_2$ is believed to damage proteins, pigments or lipids near its production site [5]. $^1O_2$ is detoxified non-enzymatically by carotenoids [6] and α-tocopherol [7]. One-electron reduction of O_2 by PSI, in turn, leads to production of O_2^- in Mehler's reaction [8], and H_2O_2 is mainly produced by dismutation of O_2^-. H_2O_2 and O_2^- are less reactive than $^1O_2$ but may induce sustained production of the extremely reactive hydroxyl radical via the Fenton reaction. Conversion of O_2^- to H_2O_2 is catalyzed by superoxide dismutase, and H_2O_2 is detoxified by catalases and peroxidases [2]. The cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) contains a superoxide dismutase [9], a catalase-peroxidase [10], five peroxiredoxins [11] and two glutathione peroxidases [12]. Glutathione accumulates in the light, and a mutant strain lacking glutathione was found to be very sensitive to oxidative stress [13]. *Synechocystis* does not contain ascorbate peroxidases which function as the principal H_2O_2 scavengers in chloroplasts [9].

Cyanobacteria also have a safe pathway for electron transfer from PSI to O_2 [14]. This Mehler-like reaction uses the flavodiiron proteins

Abbreviations: Chl, chlorophyll; CM-H_2DCFDA, 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate; CS, glucose tolerant control strain of *Synechocystis* sp. PCC 6803; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea Photosystems I, PSI; Photosystem II, PSII; ROS, reactive oxygen species; PPFD, photosynthetic photon flux density; k_{PI}, rate constant of photoinhibition

* Corresponding author at: Molecular Plant Biology, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland. Tel.: +358 2 3335797; fax: +358 2 3338075.
E-mail address: taityy@utu.fi (T. Tyystjärvi).

0005-2728/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbabio.2013.11.011

Flv1 and Flv3 in *Synechocystis* [14,15]. The Mehler-like reaction does not produce ROS and may protect PSI against production of O_2^-.

In the present paper, we use term "photoinhibition" in its narrow definition, where photoinhibition means the loss of PSII activity due to light induced damage from which recovery occurs only via the PSII repair cycle [3]. The rate of damage to PSII is directly proportional to light intensity [16], but due to efficient PSII repair mechanism, net photoinhibition only occurs in strong light. In addition to visible light, UV radiation causes photoinhibition [17–19]. Several hypotheses about the mechanism of photoinhibition have been put forward, and the role of ROS in PSII damaging reaction is still under debate [3,4,20,21]. The repair of damaged PSII requires degradation of the damaged reaction center protein D1 and synthesis of a new D1 protein [22,23]. The PSII repair cycle is sensitive to ROS because translation is vulnerable to oxidation [24].

The sigma (σ) factor mutant ΔsigCDE contains the multifunctional, stress responsive SigB as its only functional group 2 σ factor [25]. Previous studies have shown that inactivation of SigB results in a salt [26,27] and heat [28] sensitive phenotype. On the contrary, ΔsigCDE acclimates slightly better to high salt than the control strain (CS) due to up-regulation of the compatible solute producing enzyme glucosylglycerol phosphate synthase, heat shock protein HspA and carotenoids, all of which are known to increase high salt tolerance [27,29].

We have recently shown that high carotenoid content and up-regulation of Flv4-Sll0218-Flv2 proteins protect PSII against light induced damage in ΔsigCDE [30]. Results of the present work show that the resistance of ΔsigCDE against the light-induced damage does not ensure rapid growth of mutant cells in moderately high light. PSII repair cycle functioned efficiently in ΔsigCDE and PSII electron transfer reactions appeared normal. Further analyses indicated that although ΔsigCDE was resistant against PSII damage, it was vulnerable to oxidative stress. Growth of ΔsigCDE was slow when cells were exposed to methyl-viologen-induced oxidative stress (O_2^- stress). A fluorescent dye, detecting H_2O_2, hydroxyl and peroxyl radicals, and the peroxynitrite anion, showed a higher ROS level in ΔsigCDE than in CS in standard conditions, and accordingly, more protein oxidation was detected in ΔsigCDE than in CS. In addition, ΔsigCDE had a low glutathione content and a low amount of the Flv3 protein that functions in the Mehler-like reaction. The data suggest that moderate O_2^- stress, caused by a low glutathione content and down-regulation of the Mehler-like reaction, leads to up-regulation of carotenoids and Flv4, Sll0218, Flv2 proteins and low $^1O_2$ singlet oxygen content that in turn offers protection against light-induced damage of PSII.

2. Material and methods

2.1. Strains, culture conditions and determination of Chl a

The glucose tolerant control strain of *Synechocystis* sp. PCC 6803 (CS) and the σ factor inactivation strain ΔsigCDE [25] were grown in standard conditions (continuous illumination at the photosynthetic photon flux density (PPFD) of 40 μmol m^{-2} s^{-1}, 32 °C, ambient CO_2, shaking at 90 rpm, BG-11 growth medium) as described earlier [27]. For physiological measurements, OD_{730} of liquid cultures was set to 0.7 (60-mL culture in a 250-mL Erlenmeyer flask, 2.5 μg Chl *a*/mL) and cells were grown overnight (OD_{730} was ~ 1) without antibiotics. For the high light growth experiment OD_{730} was set to 0.1 (0.35 μg Chl *a*/mL), and the cells were grown under the constant PPFD of 120 μmol m^{-2} s^{-1}. For growth experiments, OD_{730} of cell cultures were set to 0.1, 4 μM methyl viologen was added, and growth was monitored for eight days. When measuring growth curves, samples of dense cultures were diluted so that OD_{730} did not exceed 0.4, and the dilutions were taken into account when the final results were calculated. Chl *a* was extracted and measured as described previously from intact cells with methanol, and from thylakoids or total protein samples with 80% acetone [27].

2.2. Cellular ROS content

Cells were grown in standard growth conditions overnight. The membrane-permeant fluorescence indicator 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, CM-H_2DCFDA (Invitrogen), was added to 1 mL of cell culture to the final concentration of 25 μM. The protocol provided by the manufacturer was followed. Briefly, after 90 min loading time in darkness at 32 °C, cells were washed twice with BG-11 and resuspended to the final volume of 0.5 mL. Then 200 μl of cell suspension were pipetted to a white 96-well microtitre plate and fluorescence from CM-H_2DCFDA and autofluorescence were measured with a Wallac Victor^2 (Perkin Elmer) using 485 nm excitation and detecting emission at 535 nm for CM-H_2DCFDA and 680 nm for autofluorescence. Then the samples were either incubated in standard conditions for 45 min or treated with high light (PPFD 750 μmol m^{-2} s^{-1}) at 32 °C for 5, 15, 30 or 45 min. Autofluorescence was used to normalize samples, and changes in CM-H_2DCFDA fluorescence were calculated to detect general ROS content (H_2O_2, hydroxyl and peroxyl radical, and peroxynitrite) of the cells.

Singlet oxygen production in intact cells was detected by His-induced chemical trapping, which is based on the removal of oxygen from the suspension due to oxidation of His by $^1O_2$. This effect can be detected and quantified by His-mediated oxygen uptake as previously described [31]. Briefly, cells were washed once and resuspended into the final concentration of 5 μg Chl/mL in fresh BG-11 medium. The light induced (PPFD of 2300 μmol m^{-2} s^{-1}) oxygen production rate was measured with and without 5 mM His using DW2 O_2 electrode (Hansatech) and the difference between the rates of light induced oxygen production with and without His was used to calculate the rate of His-induced oxygen uptake as an indication of singlet oxygen production.

2.3. Protein oxidation and glutathione measurements

Cells (30 mL; $OD_{730} = 1$) were harvested from growth conditions, or after a 30-min high-light treatment at the PPFD of 750 μmol m^{-2} s^{-1}. Total protein samples were isolated as previously described [25] except that the lysis buffer was supplemented with 50 mM dithiothreitol. Samples containing 0.1 μg Chl *a* were prepared according to Oxyblot™ Protein Oxidation Detection Kit (Millipore). Total proteins were separated by 10% NEXT GEL™ SDS-PAGE (Amresco) and the chemiluminescent HRP substrate (Immobilon™ Western, Millipore) was used for detection of carbonylated proteins.

Total glutathione concentration was measured using a colorimetric assay [32]. The glutathione concentrations of the samples were calculated from 10 min reactions using a glutathione standard.

2.4. Carotenoid and α-tocopherol measurements

Membranes were isolated as described earlier [33]. Carotenoids were extracted with methanol from membrane samples containing 9 μg Chl *a* and α-tocopherol from intact cells as described earlier [27]. After extraction, pigments and α-tocopherol were separated by HPLC. Myxoxanthophyll, zeaxanthin, echinenone, and β-carotene were detected at 490 nm, Chl *a* at 663 nm and α-tocopherol by fluorescence ($\lambda_{ex} = 295$ nm, $\lambda_{em} = 340$ nm). The relative amounts of carotenoids and α-tocopherol were calculated on Chl *a* basis.

2.5. Lipid analysis

Lipids were extracted from cells (30 mL; $OD_{730} = 1$) according to the method of Bligh and Dyer [34]. Lipid classes were separated with thin-layer chromatography and quantified by gas chromatography as described previously [35].

2.6. Photoinhibition measurements in vivo

For visible-light photoinhibition treatments, cells (30 mL in 250 mL beaker, 3.2 μg Chl *a*/mL) were illuminated with a slide projector at PPFD of 1500 μmol m^{-2} s^{-1} (saturating light) for 0, 15, 30 and 45 min at 32 °C with and without lincomycin (400 μg/mL). Addition of lincomycin prevents the function of PSII repair cycle by inhibiting translation. To test the effect of high-light pretreatment, the cells were first illuminated at PPFD of 750 μmol m^{-2} s^{-1} for 10 min, then lincomycin was added and samples were withdrawn after 0, 10 and 20 min of illumination. For UV-A/B photoinhibition treatments, cells (25 mL in 100 mL beaker, 3.2 μg Chl *a*/mL) were illuminated through a UG-11 filter (Schott) with a high-pressure Xenon lamp at 32 °C in the presence of lincomycin. The resulting light was UV light peaking at 340 nm, containing 82% of UV-A and 18% of UV-B. For UV-C photoinhibition treatments cells (25 mL in 100 mL beaker, 3.2 μg Chl *a*/mL) were illuminated at 254 nm (Vilber Lourmat VL-8-LC) in the presence of lincomycin. Aliquots of 1 mL were withdrawn and PSII activity was measured under saturating light (2000 μmol photons m^{-2} s^{-1}) with a Clark type oxygen electrode at 32 °C in the presence of an electron acceptor, 0.5 mM 2,6-dichloro-*p*-benzoquinone (DCBQ). To keep DCBQ in an oxidized form, 0.5 mM ferricyanide was added. The rate constant of photoinhibition (k_{PI}) was obtained by fitting the loss of PSII activity, measured in the presence of lincomycin, to a first-order equation.

2.7. Western blotting and pulse labeling with ^{35}[S]Met

Cells (25 mL; OD_{730} = 1; Chl *a* 3.5 μg/mL) were harvested from growth light conditions. Total protein samples were isolated as previously described [25]. Protein samples containing 0.15 μg (allophycocyanin, phycocyanin, PsaB) or 0.5 μg (CP43, Flv3) Chl *a* were solubilized for 10 min at 75 °C and separated by 10% NEXT GEL™ SDS-PAGE (Amresco) according to the manufacturer's instructions. Proteins were transferred to Immobilon-P membrane (Millipore). Antibodies against allophycocyanin (AS08277), phycocyanin (AS08278), PsaB (AS10695) and IsiA/CP43 (AS06111) were purchased from Agrisera; antibody against Flv3 was a generous gift from professor E-M. Aro. The Goat Anti-Rabbit IgG (H + L) alkaline phosphatase conjugate (Zymed) and the CDP star chemiluminescence kit (New England Biolabs) were used for detection of primary antibodies (and thus specific proteins) in Western blotting. The immunoblots were quantified with a FluorChem image analyzer (Alpha Innotech Corp.).

Cells (25 mL; OD_{730} = 1) were pulse-labeled for 10 min at the PPFD of 1500 μmol m^{-2} s^{-1} with and without 30-min pre-illumination at the same PPFD. Labeling was initiated by adding 2.5 μL of ^{35}[S] L-Met (185 MBq, Perkin Elmer). After the labeling, cold L-Met (0.4 mg/mL) was added, the samples were rapidly cooled and cells were collected by centrifugation at 4000 *g* for 5 min at 4 °C. Thylakoids were isolated as described earlier [25]. Proteins were solubilized for 10 min at 75 °C and samples containing 5 μg of Chl *a* were separated by 10% NEXT GEL™ SDS-PAGE. Proteins were transferred to Immobilon-P membrane and visualized with autoradiography. After the autoradiography, the membranes were immunodetected with a D1 antibody (Agrisera, AS06124A).

2.8. Northern blotting

For Northern blot analysis, the cells (25 mL; OD_{730} = 1) were harvested from growth conditions or after 15-min or 45-min illumination at the PPFD of 1500 μmol m^{-2} s^{-1}. Total RNA isolation and Northern hybridizations were performed as described earlier [36]. Four μg of total RNA was loaded in each well. Equal loading of the gels was confirmed by methylene blue staining. The *psbA* probe was amplified by PCR using primers 5′-TGCCTTCATCGCCGCTCCC-3′ and 5′-AGGTGCCG ATTACCCGGCCT-3′. The probes were labeled using the Prime-a-gene kit (Promega) and α-^{32}[P]dCTP 10 mCi/mL (Perkin Elmer) according to the manufacturer's instructions.

2.9. 77 K fluorescence spectroscopy

Cells were grown in standard growth conditions. The cultures were concentrated to 40 μg Chl *a*/mL and 50 μL samples were used for the measurements. Fluorescence emission spectra were measured at 77 K with an Ocean Optics S2000 spectrometer by exciting the sample with blue light obtained by filtering light from a slide projector through a 440 nm line filter (Corion, Dunedin FL, USA). The spectra were corrected by subtracting the background at 615 nm, smoothened by a moving median with a 2 nm window, and normalized by dividing by the peak value of PSI emission at 723 nm.

2.10. Decay of Chl a fluorescence yield after a single turnover flash

The F3000 fluorometer (Photon Systems Instruments, Brno, Czech Republic) was used. Before each measurement, cyanobacterial cells (1 mL samples with 20 μg Chl/mL) were incubated at 32 °C in the dark for 3 min. In some experiments, as indicated, 20 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was added at the beginning of the dark incubation. A saturating single turnover flash was fired and thereafter the fluorescence yield was measured with a series of weak probe flashes for 60 s.

2.11. Thermoluminescence measurements

Cells from 9 mL of culture (OD_{730} = 1) were collected on a glass microfiber filter 693 (VWR International). To measure the Q-band, 10 μM DCMU was added before the cells were collected. Cells on filters were dark incubated for 4 min at 32 °C before thermoluminescence was measured with a homemade luminometer [37]. The temperature was lowered to −10 °C, a 4 μs, 0.5 J Xenon flash was fired and the sample was heated at the rate of 0.5 °C/s.

3. Results and discussion

3.1. Photoinhibition resistance of ΔsigCDE does not support rapid growth in moderately high light

We have recently shown that ΔsigCDE is resistant against the damaging reaction of photoinhibition [30]. The PSII of ΔsigCDE remained more resistant against the light-induced damage than that of CS when PSII damage was measured after the cells were first pre-illuminated in high-light (Fig. 1A). However, ΔsigCDE grew slighly more slowly than CS in moderately high light, PPFD 120 μmol m^{-2} s^{-1}, (Fig. 1B) although the growth rates of ΔsigCDE and CS were similar in standard conditions [25]. These results indicate that ΔsigCDE is not generally more tolerant against high light than CS, suggesting that the advantage due to resistance of PSII against light-induced damage is lost because something else in ΔsigCDE does not properly acclimate to high light.

3.2. Carotenoid and lipid composition of ΔsigCDE

Lipids are known to play roles in photosynthetic efficiency of the cells [38], and the high carotenoid content of ΔsigCDE [30] prompted us to study the membrane composition of ΔsigCDE. The carotenoid profile of the membranes of ΔsigCDE (Fig. 2A) resembles that of the whole cells [30] indicating that extra carotenoids of ΔsigCDE are membrane bound. Analyses of membrane lipids indicated that ΔsigCDE and CS contain similar amounts of sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG), monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) (Fig. 2B). Lipid analysis showed that 16:0, the main saturated fatty acid of *Synechocystis*, was 2–3% more abundant in ΔsigCDE than in CS (Fig. 2C–F), and the unsaturated

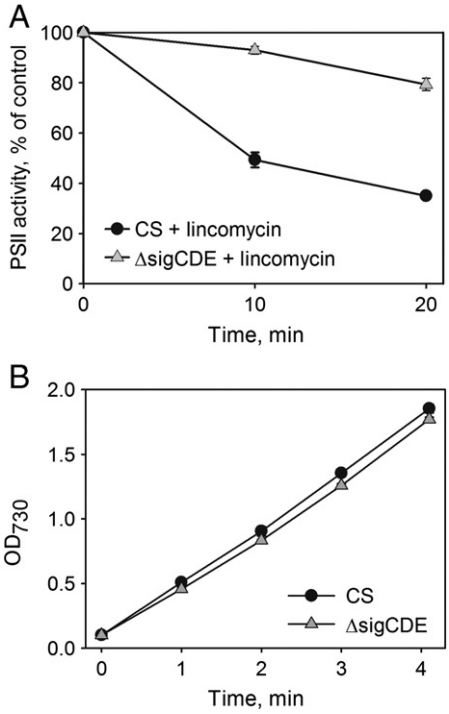

Fig. 1. The loss of PSII activity after high-light pretreatment and growth of the control (CS) and ΔsigCDE strains at moderately high light. A, The cells were first pretreated at PPFD of 750 μmol m^{-2} s^{-1} for 10 min, then lincomycin was added, and light-induced loss of PSII activity was measured. B, OD_{730} was set to 0.1 and cells were grown under the constant illumination of PPFD of 120 μmol m^{-2} s^{-1} at 32 °C in BG-11 medium pH 7.5. Three independent biological replicates were done and error bars are shown if bigger than the symbols.

fatty acid 18:3 (6,9,12) was less abundant in MGDG and DGDG in ΔsigCDE than in CS (Fig. 2C,D). Furthermore, SQDG contained 20% less 18:2 (9,12) in ΔsigCDE than in CS, but MGDG contained 8% more 18:2 (9,12) in ΔsigCDE than in CS.

3.3. Electron transfer properties of PSII are similar in CS and ΔsigCDE

Next we analyzed the electron transfer properties of ΔsigCDE. The Chl *a* content of a culture with $OD_{730} = 1$ was 3.5 μg/mL in both strains. Fluorescence emission spectra measured at 77 K indicated a slightly higher PSII peak in ΔsigCDE than in CS (Fig. 3A). However, the small difference observed at 680 to 695 nm between the strains found no confirmation when the amounts of the PsaB protein (PSI reaction center) and CP43 (a PSII protein) were compared by Western blotting (Fig. 3B). The amounts of allophycocyanin and phycocyanin were similar in both strains (Fig. 3B).

The decay of Chl *a* fluorescence yield after a single turnover flash showed that electron transfer from Q_A to Q_B followed similar kinetics in both strains (Fig. 3C). The B and Q bands of thermoluminescence (Fig. 3D) were similar in both strains, and the decay of Chl *a* fluorescence yield, measured in the presence of DCMU was essentially identical in both strains (Fig. 3C), indicating that charge recombination reactions of PSII occurred similarly in both strains. These measurements showed that PSII electron transfer reactions function normally in ΔsigCDE.

3.4. PSII repair cycle functions normally in ΔsigCDE

We have previously shown that the efficiency of the PSII repair cycle is reduced in the ΔsigBD strain because *psbA* genes encoding the D1 protein are not up-regulated as efficiently as in CS [39]. The PSII repair cycle in ΔsigCDE was studied at the PPFD of 1500 μmol m^{-2} s^{-1}.

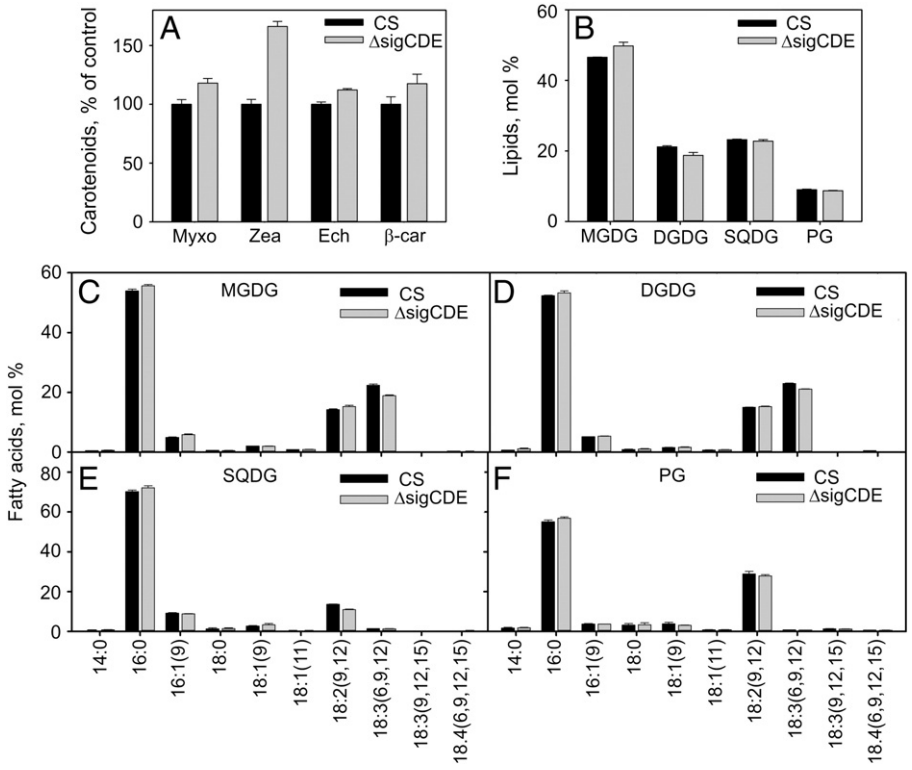

Fig. 2. Carotenoid and lipid composition of membranes. A, Carotenoids of CS and ΔsigCDE were extracted from the membranes. The cells were grown in BG-11 medium at 32 °C in continuous light at the PPFD of 40 μmol m^{-2} s^{-1} (standard conditions). Myxo, myxoxanthophyll; Zea, zeaxanthin; Ech, echinenone; β-car, β-carotene. B, Composition of lipids in CS and ΔsigCDE. Lipids were extracted from the cells grown in standard conditions. C–F, Fatty acid composition of each lipid class. Each bar represents the mean of three independent experiments, and the error bars denote SE.

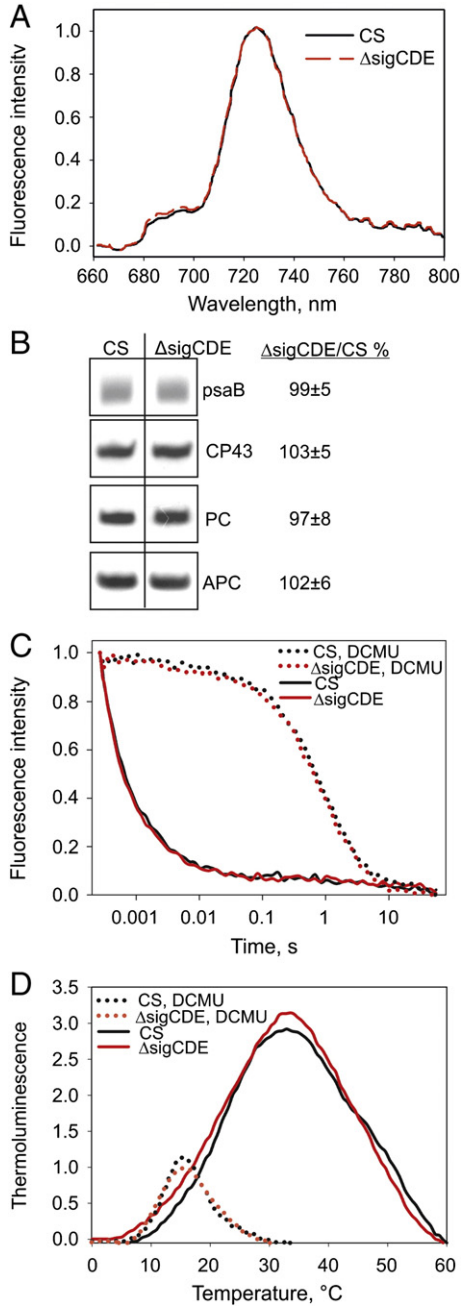

Fig. 3. Stoichiometry of photosystems in the control and ΔsigCDE strains and PSII electron transfer reactions. A, Blue-light excited fluorescence emission spectra of the cells at 77 K. The spectra were normalized to the PSI emission peak at 723 nm. Each spectrum represents an average of three independent measurements. B, The PsaB, CP43, phycocyanin (PC) and allophycocyanin (APC) proteins were detected by Western blotting. Total proteins were isolated from the cells grown in standard growth conditions and equal protein samples were separated by SDS-PAGE. Representative blots of three independent measurements are shown. C, Decay of Chl *a* fluorescence yield after a single turnover flash measured at 32 °C in the absence (solid lines) and presence (dashed lines) of DCMU. The cells were preincubated in the dark and DCMU was added at the beginning of the dark incubation. D, Thermoluminescence glow curves measured in the absence (solid lines) and presence (dashed lines) of DCMU. Cells were collected to a glass fiber filter, incubated in the dark for 3 min and cooled to −10 °C. A. single turnover flash was fired and thereafter the samples were heated at 0.5 °C s^{-1}. Each curve is average of three independent experiments.

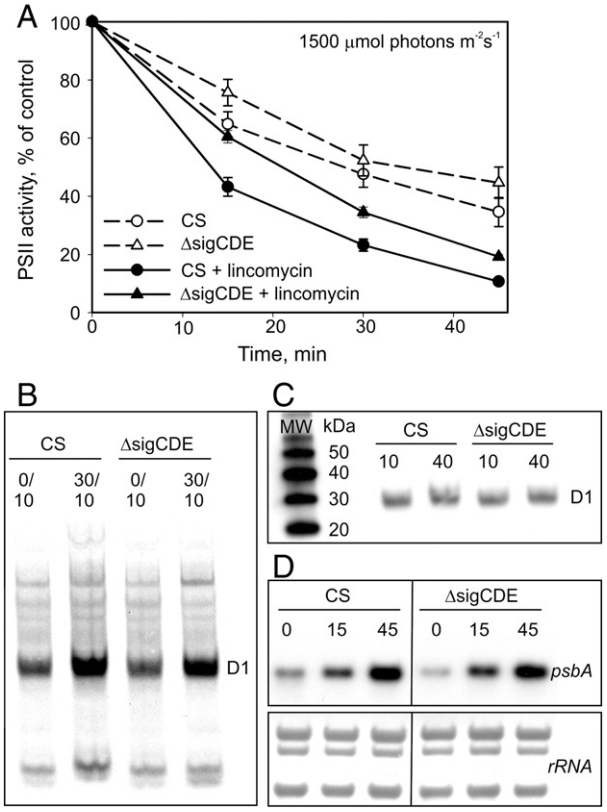

Fig. 4. PSII photoinhibition and repair cycle in the control and ΔsigCDE strains. A, Loss of oxygen evolution when cells were illuminated at the PPFD of 1500 μmol m^{-2} s^{-1} with (solid lines) and without (dashed lines) lincomycin. Light-saturated PSII activity was measured using DCBQ as an electron acceptor. Each data point represents the mean of at least three independent biological replicates, and the error bars denote SE. B, Translational activity in high light. Cells were pre-illuminated for 0 and 30 min at the PPFD of 1500 μmol m^{-2} s^{-1}, and then pulse labeled with L-[^{35}S]Met for 10 min at the PPFD of 1500 μmol m^{-2} s^{-1}. Proteins of isolated thylakoid membranes containing 5 μg of Chl *a* were separated by SDS-PAGE and visualized with autoradiography. C, The amount of D1 protein detected by Western blotting. D, The *psbA* transcripts detected by Northern blotting after 0, 15 and 45 min treatments at the PPFD of 1500 μmol m^{-2} s^{-1}. Equal loading and even transfer of RNAs were verified by methylene blue staining of the membrane. Representative blots of three independent measurements are shown in B–D.

When cells of CS and ΔsigCDE were illuminated with white light, the PSII activity of ΔsigCDE remained slightly higher than that of the control strain (Fig. 4A) and in accordance with our previous results the PSII damaging reaction, measured in presence of a translation inhibitor, occurred circa 30% more slowly in ΔsigCDE than in CS, as the rate constant of photoinhibition was 0.052 ± 0.002 min^{-1} in CS and 0.035 ± 0.001 min^{-1} in ΔsigCDE (Fig. 4A).

To measure *de novo* synthesis of the D1 protein, cells were pulse labeled with radioactive methionine. Exposure to high light for 10 min was either performed immediately after transfer of the cells to high light or after a 30-min pre-illumination with the same high light. The D1 protein was the most efficiently produced protein already at the beginning of the high-light treatment, and enhancement of D1 synthesis after pre-illumination was similar in CS and ΔsigCDE (Fig. 4B). Western blot analysis indicated that the total amount of the D1 protein remained constant throughout the high-light treatment in both strains (Fig. 4C), and Northern blot analysis showed that high-light induction of *psbA* mRNAs occurred similarly in both strains (Fig. 4D). These results demonstrate that SigB as the only remaining group 2 σ factor is sufficient to maintain an efficient PSII repair cycle.

3.5. The ΔsigCDE strain is sensitive to oxidative stress

In addition to PSII damage, illumination with high light enhances ROS production and oxidative stress. The sensitivity of ΔsigCDE to O_2^- and to H_2O_2 originating from O_2^- was tested by growing cells in the presence of methyl viologen, a PSI electron acceptor that produces O_2^-

upon oxidation [40]. In the presence of 4 μM methyl viologen, ΔsigCDE grew more slowly than CS (Fig. 5A), indicating that ΔsigCDE is sensitive to methyl-viologen-induced O_2^- stress.

To estimate the cellular ROS content of the strains, cells were first loaded in darkness with the fluorescence dye CM-H_2DCFDA (detects H_2O_2, hydroxyl and peroxyl radicals, and the peroxynitrite) and then accumulation of ROS was followed in standard growth conditions (40 μmol photons m^{-2} s^{-1}) for 45 min or in high light (750 μmol photons m^{-2} s^{-1}) for 5,15,30 or 45 min. In standard conditions, ΔsigCDE had a 33% higher ROS content than CS (Fig. 5B). High-light treatment induced increase of ROS content in both strains, but the ROS content increased more rapidly and ended up to a higher level in ΔsigCDE than in CS (Fig. 5B). In accordance with a higher ROS level in the ΔsigCDE strain, an immunological test for carbonylated proteins detected more oxidized proteins in ΔsigCDE than in CS in standard growth conditions (Fig. 5C) and a 30-min high-light treatment increased protein oxidation in both strains (Fig. 5C). The $^1O_2$ content of cells was measured by histidine-mediated chemical trapping [31]. In this method, a good $^1O_2$ acceptor, His, reacts with $^1O_2$ and removes dissolved O_2 from cell suspension in $^1O_2$-concentration dependent manner [31]. The extent of O_2 uptake, which is induced by the presence of His is proportional with the amount of produced $^1O_2$. In contrast to the other ROS species, the $^1O_2$ content of ΔsigCDE cells was 65% lower than that of CS (Fig. 5D). Taken together, the results show that ΔsigCDE suffers from mild oxidative stress in our standard growth conditions, and higher accumulation of some ROS species and protein damage were detected in ΔsigCDE than in CS while the amount of $^1O_2$ was low in ΔsigCDE.

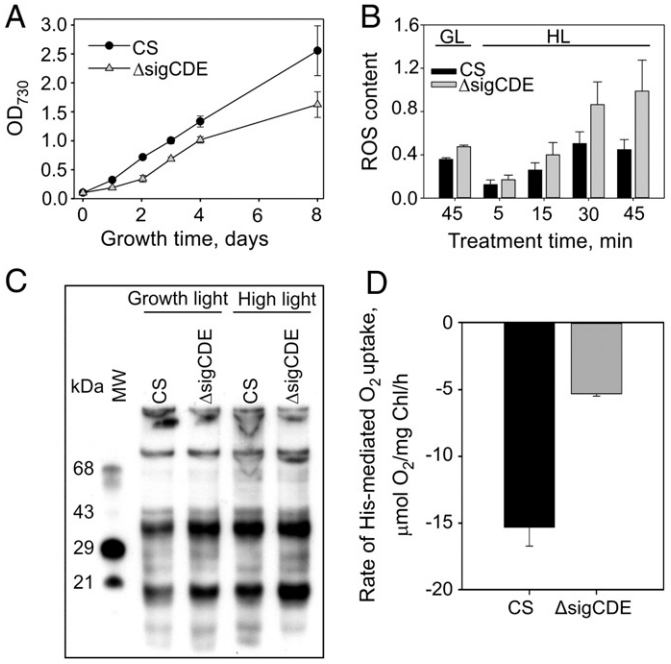

Fig. 5. Sensitivity of ΔsigCDE to reactive oxygen species. A, Growth of the control (CS) and ΔsigCDE strains in BG-11 medium supplemented with 4 μM methyl viologen at 32 °C in continuous light, PPFD 40 μmol m^{-2} s^{-1}. B, Relative amounts of ROS in CS and ΔsigCDE strains after 45 min in standard growth light (GL) conditions (at the PPFD of 40 μmol m^{-2} s^{-1}) and after illumination for 5, 15, 30 and 45 min at the PPFD of ~750 μmol m^{-2} s^{-1} (HL) using the membrane-permeant fluorescence indicator CM-H_2DCFDA. C, Protein oxidation levels in CS and ΔsigCDE strains were detected using Oxyblot™ Kit. Proteins were isolated from cells grown in growth light and after a 30 min high light treatment at the PPFD of 750 μmol m^{-2} s^{-1}. A representative plot of three independent measurements is shown. D, Singlet oxygen production in ΔsigCDE and CS. $^1O_2$ production was measured by His-mediated oxygen uptake in BG-11 medium. The extent of O_2 uptake, which is induced by the presence of His is proportional with the amount of produced $^1O_2$. Each bar and data point represents the mean of three independent experiments, and the error bars denote SE.

3.6. Protectants against oxidative stress in the ΔsigCDE strain

The Mehler-like reaction in which flavodiiron proteins reduce O_2 to water without producing ROS can be an important protection mechanism against formation of O_2^- in cyanobacteria [14,15]. In *Synechocystis*, the Flv3/Flv1 heterodimer functions in the Mehler-like reaction [14]. Western blot analysis showed that the amount of the Flv3 protein was reduced by one third in ΔsigCDE compared to CS (Fig. 6A). Down-regulation of the Flv3 protein in ΔsigCDE was due to a low level of *flv3* mRNA (Supplementary Table S1). It has been estimated that up to 20% of electrons might pass from PSI to O_2 via the Flv1/Flv3 dimer [15]. Since normal PSII and PSI contents and electron transfer reactions were detected in ΔsigCDE, it might be that a low Flv3 content slows down the Mehler-like reaction in ΔsigCDE causing a considerable flow of electrons to true Mehler's reaction and therefore leading to high production of O_2^-. A slow Mehler-like reaction may be one of the reasons behind the low methyl viologen tolerance of ΔsigCDE (Fig. 5A).

Enzymes detoxifying O_2^- and its reaction product H_2O_2, including superoxide dismutase, catalase, glutathione peroxidases and peroxiredoxins, were expressed similarly in ΔsigCDE and CS at the mRNA level (Supplementary Table S1) suggesting that the higher ROS content of ΔsigCDE does not induce mechanisms known to detoxify O_2^- and its derivatives. Cyanobacteria contain millimolar levels of glutathione that functions as an antioxidant and maintains cellular redox balance [41]. ΔsigCDE contained approximately 20% less glutathione than CS (Fig. 6B). A *Synechocystis* mutant lacking glutathione synthase (*gshB, slr1238*) has been shown to be extremely sensitive to H_2O_2, methyl viologen and to the $^1O_2$ sensitizing dye Rose Bengal [13], indicating that glutathione protects against all major ROS in cyanobacteria. Thus, the symptoms of oxidative stress in ΔsigCDE may partially depend on the low glutathione content of ΔsigCDE.

Glutaredoxin and thioredoxins are involved in glutathione-dependent mechanisms that maintain cellular redox balance in cyanobacteria [42,43]. The STXQ mutant of *Synechocystis,* lacking thioredoxin Q, is sensitive to H_2O_2 [44] and a deletion mutant lacking NADPH thioredoxin reductase (NTR) is sensitive to methyl viologen [45]. The mRNA levels of *trxQ* (*slr0233*) and *NTR* (*slr0600*) were down regulated in the ΔsigCDE (Supplemental Table S1), suggesting that low glutathione content is not the only defect in cellular redox balancing systems in ΔsigCDE. Defects in cellular redox balancing may contribute to the slow growth in the presence of methyl viologen (Fig. 5A), the high content of O_2^- and related ROS species (Fig. 5B) and the high protein oxidation in ΔsigCDE (Fig. 5C).

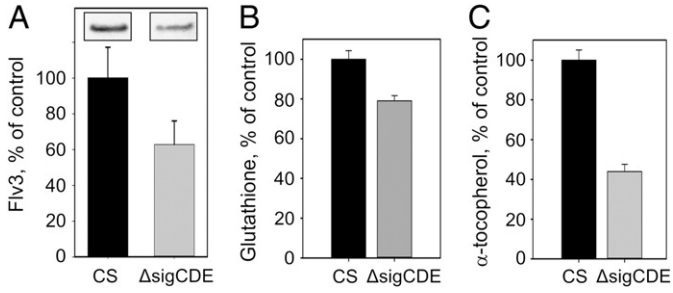

Fig. 6. Protective mechanisms against reactive oxygen species in ΔsigCDE. A, The content of Flv3 protein in CS and ΔsigCDE strains. Total proteins were isolated from the cells grown in growth light conditions. Equal protein samples were separated by SDS-PAGE, Flv3 protein was detected with Western blotting and quantified. B, Total glutathione content in CS and ΔsigCDE strains in growth light conditions. C, α-tocopherol content of CS and ΔsigCDE in standard conditions. Relative amount of α-tocopherol was calculated on Chl *a* basis. Each bar in A–C represents the mean value of three independent biological replicates, and the error bars denote SE.

3.7. Role of $^1O_2$ in photoinhibition

$^1O_2$ is mainly quenched and scavenged non-enzymatically by carotenoids and α-tocopherol. The carotenoid content of ΔsigCDE was high (Fig. 2A) but the amount of α-tocopherol in ΔsigCDE was only half of that measured from the control strain (Fig. 6C). This was most probably due to low expression of genes *slr1736*, *slr1737* and *slr0089*, encoding homogentisate phytyltransferase, tocopherol cyclase and γ-tocopherol methyltransferase, respectively, all involved in the synthesis of α-tocopherol (Supplementary Table S1).

Synechocystis and plant mutants lacking α-tocopherol have similar rates of the damaging reaction of photoinhibition as the respective wild types, but the mutants have deficiencies in the PSII repair cycle [7]. Although the α-tocopherol content of ΔsigCDE was lower than in CS (Fig. 6C), the PSII repair cycle remained fully functional (Fig. 4). Obviously, both carotenoids and α-tocopherol can protect the PSII repair cycle against oxidative damage. Carotenoids and α-tocopherol have been suggested to play overlapping roles in the protection against lipid peroxidation [46] and photo-oxidative stress [47].

Our results suggest that the resistance of ΔsigCDE against the light-induced damage to PSII might be related to low $^1O_2$ level of the strain. To further test this hypothesis, we measured the damage of PSII in two different UV ranges. The UV-A/B light (82% of UV-A and 18% of UV-B) is supposed to induce some formation of $^1O_2$ while short wavelength UV-C is not [48]. The ΔsigCDE strain lost PSII activity more slowly than CS when cells were illuminated in UV-A/B light in the presence of lincomycin (Fig. 7A) while the PSII damage rates of the strains were identical under UV-C light (Fig. 7B). These results support the idea that efficient protection against $^1O_2$ in ΔsigCDE is the main reason for the PSII damage resistant phenotype of this strain in visible light. It is possible that high carotenoid content contributes to the photoinhibition resistance of ΔsigCDE also in UV-A/B light, but involvement of other protective mechanisms, like up-regulation of the *flv4-sll0218-flv2* operon [30], is possible as well. In accordance with our results, a zeaxanthin-overproducing strain of a green alga has been shown to be resistant to the damaging reaction of photoinhibition [49]. Chlorophyll fluorescence measurements (Fig. 3) excluded differences in charge recombination between the electron acceptor Q_A and S_2/S_3 states of the oxygen-evolving complex, implying that the rate of $^1O_2$ production by the slow charge recombination mechanism was similar in ΔsigCDE and CS. Differences in other $^1O_2$ production mechanisms are unlikely, as PSII is equally active in ΔsigCDE and CS. The manganese mechanism of photoinhibition [3] suggests that charge recombination reactions lead to production of $^1O_2$ when the oxygen-evolving manganese complex does not provide electrons, whereas in the acceptor side mechanism [4,50] charge recombination reactions are caused by inhibition of electron transfer at the acceptor side of PSII. The recent finding that the rate of $^1O_2$ production in cyanobacteria is directly proportional to light intensity [31] may suggest a direct link between $^1O_2$ production and the light-induced damage of PSII, as also the rate of photoinhibition is directly proportional to light intensity [16]. The present data do not allow judgment between the different mechanisms that trigger $^1O_2$ production.

4. Conclusions

The group 2 σ factor mutant ΔsigCDE strain having SigB as an only group 2 σ factor, shows an interesting phenotype, being more sensitive to oxidative stress (Fig. 5) but more resistant to photoinhibition of PSII (Figs. 1, 4 and 7). Fig. 8 shows a summary of mechanisms that are involved in ROS metabolism and are up or down-regulated in ΔsigCDE. Down-regulation of the Mehler-like reaction (Flv3 protein) and redox balancing systems (low amount of glutathione and *trxQ* and *NTR)* lead to oxidative stress in ΔsigCDE which up-regulates production of photoprotective carotenoids and Flv4, sll0218, Flv2 proteins. Efficient quenching of singlet oxygen in ΔsigCDE protects PSII against light induced damage. Carotenoids and α-tocopherol show redundant functions in protecting the PSII repair cycle against oxidative damage.

The high resistance of ΔsigCDE against the damaging reaction of photoinhibition indicates that photoinhibition tolerance can be achieved by up-regulating protective mechanisms by genetic modifications. In ΔsigCDE inactivation of the three other group 2 σ factors enhances the probability that the stress responsive SigB gets recruited

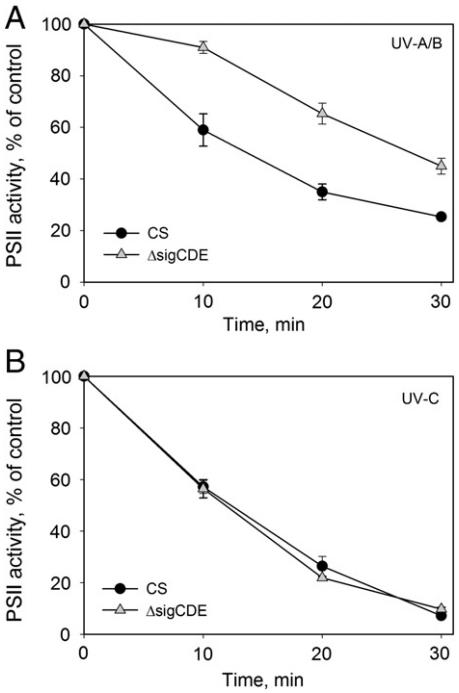

Fig. 7. Loss of PSII activity after UV light illumination in the presence of lincomycin. Cells were illuminated either with UV-A/B (peaking at 340 nm, 82% UV-A and 18% UV-B) or UV-C (254 nm) light. Each data point is the mean of three independent biological replicates, and the error bars denote SE.

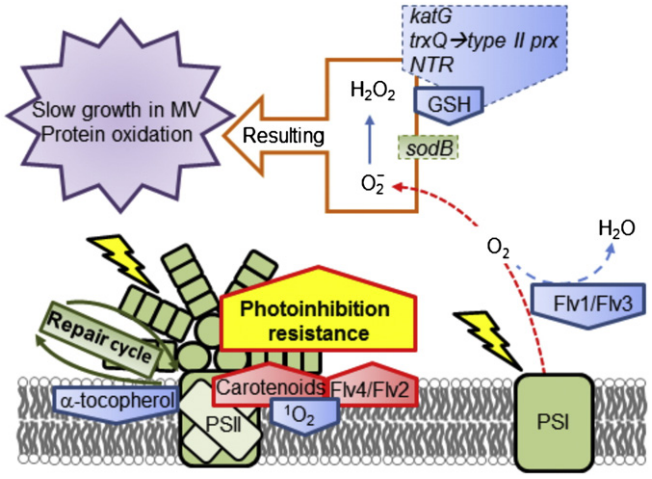

Fig. 8. Differently regulated protective mechanisms in ΔsigCDE results photoinhibition resistant, but ROS sensitive, phenotype. Red indicates up regulated and blue down regulated mechanisms in ΔsigCDE. Blue boxes with dashed line show down regulation of the gene expression in mRNA level. Green color indicates similar function in ΔsigCDE and CS.

by the RNA polymerase core and induces a photoinhibition resistant phenotype by reducing the $^1O_2$ content of the cells. However, inactivated SigC, SigD and/or SigE factors were found to be important for normal functioning of redox balancing mechanisms and protection against other ROS than $^1O_2$. The high light sensitivity of ΔsigD [26,39] suggests that SigD might have a central role controlling ROS protection and redox balancing mechanisms.

According to our results, resistance against the light induced damage to PSII alone does not lead to high light tolerance of cyanobacteria. To obtain a high capacity for biomass production in high light, cyanobacteria need to have efficient protection against O_2^- related oxidative stress. Our results demonstrate that tolerance against light induced damage of PSII and overall tolerance to oxidative stress can be separated in cyanobacteria, and that the main protective mechanisms are different.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbabio.2013.11.011.

Acknowledgements

This work was supported by the Academy of Finland. I. Vass and A. Rehman were supported by the Hungarian Granting Agency OTKA K-101433 and by the TÁMOP-4.2.2.A-11/1/KONV-2012-0047 project. Professor E.-M. Aro is thanked for providing Flv3 antibody and Dr. Lasse Välimaa for providing Wallac Victor.

References

[1] M. Muramatsu, Y. Hihara, Acclimation to high-light conditions in cyanobacteria: from gene expression to physiological responses, J. Plant Res. 125 (2012) 11–39.
[2] A. Latifi, M. Ruiz, C. Zhang, Oxidative stress in cyanobacteria, FEMS Microbiol. Rev. 33 (2009) 258–278.
[3] E. Tyystjärvi, Photoinhibition of photosystem II, Int. Rev. Cell. Mol. Biol. 300 (2013) 243–303.
[4] I. Vass, Role of charge recombination processes in photodamage and photoprotection of the photosystem II complex, Physiol. Plant. 142 (2011) 6–16.
[5] C. Triantaphylides, M. Havaux, Singlet oxygen in plants: production, detoxification and signaling, Trends Plant Sci. 14 (2009) 219–228.
[6] L. Schäfer, A. Vioque, G. Sandmann, Functional *in situ* evaluation of photosynthesis-protecting carotenoids in mutants of the cyanobacterium *Synechocystis* PCC6803, J. Photochem. Photobiol. B 78 (2005) 195–201.
[7] S. Inoue, K. Ejima, E. Iwai, H. Hayashi, J. Appel, E. Tyystjärvi, N. Murata, Y. Nishiyama, Protection by α-tocopherol of the repair of photosystem II during photoinhibition in *Synechocystis* sp. PCC 6803, Biochim. Biophys. Acta 1807 (2011) 236–241.
[8] T. Fujii, E. Yokoyama, K. Inoue, H. Sakurai, The sites of electron donation of Photosystem-I to methylviologen, Biochim. Biophys. Acta 1015 (1990) 41–48.
[9] M. Nakao, S. Okamoto, M. Kohara, T. Fujishiro, T. Fujisawa, S. Sato, S. Tabata, T. Kaneko, Y. Nakamura, CyanoBase: the cyanobacteria genome database update 2010, Nucleic Acids Res. 38 (2010) D379–D381.
[10] M. Tichy, W. Vermaas, *In vivo* role of catalase-peroxidase in *Synechocystis* sp. strain PCC 6803, J. Bacteriol. 181 (1999) 1875–1882.
[11] T. Stork, K.P. Michel, E.K. Pistorius, K.J. Dietz, Bioinformatic analysis of the genomes of the cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 for the presence of peroxiredoxins and their transcript regulation under stress, J. Exp. Bot. 56 (2005) 3193–3206.
[12] A. Gaber, M. Tamoi, T. Takeda, Y. Nakano, S. Shigeoka, NADPH-dependent glutathione peroxidase-like proteins (Gpx-1, Gpx-2) reduce unsaturated fatty acid hydroperoxides in *Synechocystis* PCC 6803, FEBS Lett. 499 (2001) 32–36.
[13] J.C. Cameron, H.B. Pakrasi, Essential role of glutathione in acclimation to environmental and redox perturbations in the cyanobacterium *Synechocystis* sp. PCC 6803, Plant Physiol. 154 (2010) 1672–1685.
[14] Y. Helman, D. Tchernov, L. Reinhold, M. Shibata, T. Ogawa, R. Schwarz, I. Ohad, A. Kaplan, Genes encoding A-type flavoproteins are essential for photoreduction of O_2 in cyanobacteria, Curr. Biol. 13 (2003) 230–235.
[15] Y. Allahverdiyeva, M. Ermakova, M. Eisenhut, P. Zhang, P. Richaud, M. Hagemann, L. Cournac, E.-M. Aro, Interplay between flavodiiron proteins and photorespiration in *Synechocystis* sp. PCC 6803, J. Biol. Chem. 286 (2011) 24007–24014.
[16] E. Tyystjärvi, E.-M. Aro, The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 2213–2218.
[17] M. Hakala, I. Tuominen, M. Keränen, T. Tyystjärvi, E. Tyystjärvi, Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of Photosystem II, Biochim. Biophys. Acta 1706 (2005) 68–80.
[18] T. Tyystjärvi, I. Tuominen, M. Herranen, E.-M. Aro, E. Tyystjärvi, Action spectrum of *psbA* gene transcription is similar to that of photoinhibition in *Synechocystis* sp. PCC 6803, FEBS Lett. 516 (2002) 167–171.
[19] C. Sicora, Z. Mate, I. Vass, The interaction of visible and UV-B light during photodamage and repair of Photosystem II, Photosynth. Res. 75 (2003) 127–137.
[20] Y. Nishiyama, S.I. Allakhverdiev, N. Murata, Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II, Physiol. Plant. 142 (2011) 35–46.
[21] Y. Nishiyama, S.I. Allakhverdiev, N. Murata, A new paradigm for action of reactive oxygen species in the photoinhibition of photosystem II, Biochim. Biophys. Acta 1757 (2006) 742–749.
[22] Y. Nishiyama, H. Yamamoto, S.I. Allakhverdiev, M. Inaba, A. Yokota, N. Murata, Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery, EMBO J. 20 (2001) 5587–5594.
[23] P. Mulo, C. Sicora, E.-M. Aro, Cyanobacterial *psbA* gene family: optimization of oxygenic photosynthesis, Cell. Mol. Life Sci. 66 (2009) 3697–3710.
[24] K. Kojima, M. Oshita, Y. Nanjo, K. Kasai, Y. Tozawa, H. Hayashi, Y. Nishiyama, Oxidation of elongation factor G inhibits the synthesis of the D1 protein of photosystem II, Mol. Microbiol. 65 (2007) 936–947.
[25] M. Pollari, S. Rantamäki, T. Huokko, A. Kårlund-Marttila, V. Virjamo, E. Tyystjärvi, T. Tyystjärvi, Effects of deficiency and overdose of group 2 σ factors in triple inactivation strains of *Synechocystis* sp. strain PCC 6803, J. Bacteriol. 193 (2011) 265–273.
[26] M. Pollari, L. Gunnelius, I. Tuominen, V. Ruotsalainen, E. Tyystjärvi, T. Salminen, T. Tyystjärvi, Characterization of single and double inactivation strains reveals new physiological roles for group 2 sigma factors in the cyanobacterium *Synechocystis* sp PCC 6803, Plant Physiol. 147 (2008) 1994–2005.
[27] H.-L. Nikkinen, K. Hakkila, L. Gunnelius, T. Huokko, M. Pollari, T. Tyystjärvi, The SigB σ factor regulates multiple salt acclimation responses of the cyanobacterium *Synechocystis* sp. PCC 6803, Plant Physiol. 158 (2012) 514–523.
[28] I. Tuominen, M. Pollari, E. Tyystjärvi, T. Tyystjärvi, The SigB σ factor mediates high-temperature responses in the cyanobacterium *Synechocystis* sp. PCC6803, FEBS Lett. 580 (2006) 319–323.
[29] T. Tyystjärvi, T. Huokko, S. Rantamäki, E. Tyystjärvi, Impact of different group 2 sigma factors on light use efficiency and high salt stress in the cyanobacterium *Synechocystis* sp. PCC 6803, PLoS One 8 (2013) e63020.
[30] K. Hakkila, T. Antal, L. Gunnelius, J. Kurkela, H.C.P. Matthijs, E. Tyystjärvi, T. Tyystjärvi, Group 2 sigma factor mutant ΔsigCDE of the cyanobacterium *Synechocystis* sp. PCC 6803 reveals functionality of both carotenoids and flavodiiron proteins in photoprotection of photosystem II, Plant Cell Physiol. 54 (2013) 1780–1790.
[31] A.U. Rehman, K. Cser, L. Sass, I. Vass, Characterization of singlet oxygen production and its involvement in photodamage of Photosystem II in the cyanobacterium *Synechocystis* PCC 6803 by histidine-mediated chemical trapping, Biochim. Biophys. Acta 1827 (2013) 689–698.
[32] C.W.I. Owens, R.V. Belcher, A colorimetric micro-method for determination of glutathione, Biochem. J. 94 (1965) 705–711.
[33] M. Hagio, Z. Gombos, Z. Várkonyi, K. Masamoto, N. Sato, M. Tsuzuki, H. Wada, Direct evidence for requirement of phosphotidylglycerol in photosystem II of photosynthesis, Plant Physiol. 124 (2000) 795–804.
[34] E. Bligh, W. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.
[35] H. Wada, N. Murata, *Synechocystis* PCC6803 mutants defective in desaturation of fatty acids, Plant Cell Physiol. 30 (1989) 971–978.
[36] T. Tyystjärvi, M. Herranen, E.-M. Aro, Regulation of translation elongation in cyanobacteria: membrane targeting of the ribosome nascent-chain complexes controls the synthesis of D1 protein, Mol. Microbiol. 40 (2001) 476–484.
[37] E. Tyystjärvi, S. Rantamäki, J. Tyystjärvi, Connectivity of photosystem II is a physical basis of retrapping in photosynthetic thermoluminescence, Biophys. J. 96 (2009) 3735–3743.
[38] N. Mizusawa, H. Wada, The role of lipids in photosystem II, Biochim. Biophys. Acta 1817 (2012) 194–208.
[39] M. Pollari, V. Ruotsalainen, S. Rantamäki, E. Tyystjärvi, T. Tyystjärvi, Simultaneous inactivation of sigma factors B and D interferes with light acclimation of the cyanobacterium *Synechocystis* sp. strain PCC 6803, J. Bacteriol. 191 (2009) 3992–4001.
[40] D.J. Thomas, T.J. Avenson, J.B. Thomas, S.K. Herbert, A cyanobacterium lacking iron superoxide dismutase is sensitized to oxidative stress induced with methyl viologen but is not sensitized to oxidative stress induced with norflurazon, Plant Physiol. 116 (1998) 1593–1602.
[41] K. Suginaka, K. Yamamoto, H. Ashida, Y. Sawa, H. Shibata, Effect of intracellular glutathione on heat-induced cell death in the cyanobacterium, *Synechocystis* PCC 6803, Biosci. Biotechnol. Biochem. 63 (1999) 1112–1115.
[42] F.J. Florencio, M.E. Pérez-Pérez, L. López-Maury, A. Mata-Cabana, M. Lindahl, The diversity and complexity of the cyanobacterial thioredoxin systems, Photosynth. Res. 89 (2006) 157–171.
[43] S.D. Lemaire, The glutaredoxin family in oxygenic photosynthetic organisms, Photosynth. Res. 79 (2004) 305–318.
[44] M.E. Pérez-Pérez, E. Martín-Figueroa, F.J. Florencio, Photosynthetic regulation of the cyanobacterium *Synechocystis* sp. PCC 6803 thioredoxin system and functional analysis of TrxB (Trx x) and TrxQ (Trx y) thioredoxins, Mol. Plant 2 (2009) 270–283.
[45] S. Hishiya, W. Hatakeyama, Y. Mizota, N. Hosoya-Matsuda, K. Motohashi, M. Ikeuchi, T. Hisabori, Binary reducing equivalent pathways using NADPH-thioredoxin reductase and ferredoxin-thioredoxin reductase in the cyanobacterium *Synechocystis* sp. strain PCC 6803, Plant Cell Physiol. 49 (2008) 11–18.

[46] H. Maeda, Y. Sakuragi, D.A. Bryant, D. Dellapenna, Tocopherols protect *Synechocystis* sp. strain PCC 6803 from lipid peroxidation, Plant Physiol. 138 (2005) 1422–1435.
[47] Z. Li, J.D. Keasling, K.K. Niyogi, Overlapping photoprotective function of vitamin E and carotenoids in *Chlamydomonas*, Plant Physiol. 158 (2012) 313–323.
[48] C. Barta, T. Kalai, K. Hideg, I. Vass, E. Hideg, Differences in the ROS-generating efficacy of various ultraviolet wavelengths in detached spinach leaves, Funct. Plant Biol. 31 (2004) 23–28.
[49] P. Jahns, B. Depka, A. Trebst, Xanthophyll cycle mutants from *Chlamydomonas reinhardtii* indicate a role for zeaxanthin in the D1 protein turnover, Plant Physiol. Biochem. 38 (2000) 371–376.
[50] I. Vass, S. Styring, T. Hundal, A. Koivuniemi, E.-M. Aro, B. Andersson, Reversible and irreversible intermediates during photoinhibition of photosystem II: stable reduced Q_A species promote chlorophyll triplet formation, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 1408–1412.