Biochimica et Biophysica Acta 1817 (2012) 1306–1313

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Reactive oxygen intermediates produced by photosynthetic electron transport are enhanced in short-day grown plants ☆

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ARTICLE INFO

Article history:
Received 13 October 2011
Received in revised form 10 November 2011
Accepted 16 November 2011
Available online 7 December 2011

Keywords:
Photoperiod
Reactive oxygen species
Spin trap
EPR spectroscopy
Pseudocyclic electron transport

ABSTRACT

Leaves of tobacco plants grown in short days (8 h light) generate more reactive oxygen species in the light than leaves of plants grown in long days (16 h light). A two fold higher level of superoxide production was observed even in isolated thylakoids from short day plants. By using specific inhibitors of photosystem II and of the cytochrome b_6f complex, the site of O_2 reduction could be assigned to photosystem I. The higher rate of O_2 reduction led to the formation of a higher proton gradient in thylakoids from short day plants. In the presence of an uncoupler, the differences in O_2 reduction between thylakoids from short day and long day plants were abolished. The pigment content and the protein content of the major protein complexes of the photosynthetic electron transport chain were unaffected by the growth condition. Addition of NADPH, but not of NADH, to coupled thylakoids from long day plants raised the level of superoxide production to the same level as observed in thylakoids from short day plants. The hypothesis is put forward that the binding of an unknown protein permits the higher rate of pseudocyclic electron flow in thylakoids from short-day grown plants and that this putative protein plays an important role in changing the proportions of linear, cyclic and pseudocyclic electron transport in favour of pseudocyclic electron transport. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Articifical.

1. Introduction

Plant development is controlled by numerous external factors. Among those, the quantity and quality of light and the length of the daily light period are very important. The first scientific description of the effects of light on plant development was performed in the 19th century by Julius Sachs [1]. Aside from the influence of photoreceptors (phytochromes, cryptochromes) on the development, photosynthesis as energy source plays a crucial role in the normal development. There are large metabolic changes in plants grown in (or shifted to) different light periods. A main focus in research during the last 30 years was on starch accumulation and the partitioning of assimilated CO_2 in soluble sugars and starch (e.g. [2–6]). The partitioning of photosynthate into chloroplast starch is altered according to the length of the daily photosynthetic photoperiod in many species [3]. It was shown by Gibon et al. [7] that starch synthesis is 4-fold stimulated in *Arabidopsis* wt in short day conditions because of sugar depletion during the long night and rapid sugar accumulation during the start of the light period. Changes in the length of the light period evoke large changes on the transcriptome level. Thimm et al. [8] reported that 6 h extension of the night leads to a decreased level of transcripts of hundreds of genes involved in anabolic pathways and to an increased level of transcripts of hundreds of genes involved in catabolic pathways. In addition, many changes occurred in the expression of genes involved in hormone synthesis, sensing, regulation of transcription and protein modification.

There are several reports in the literature that mutants affected in very different genes show a morphologically visible phenotype only when grown under short day conditions while they are indistinguishable from the wt when grown under long day conditions. In the following some references are given to publications on mutants defect in the plastidial ATP/ADP transporters [9], in the mitochondrial AtFTsH4 protease [10] and in the chloroplast NADPH-thioredoxin reductase [11]. Some of the mutants affected in short day conditions are defective in genes involved in detoxifying reactive oxygen species (ROS). It has been shown, for example, that the peptide methionine sulfoxide reductase 2 prevents oxidative damage in long nights [12]. Mutants defective in this gene showed a higher amount of ROS in the leaves. In a catalase-deficient mutant (cat2) the transcript level of H_2O_2-inducible genes was strongly induced when grown in short day conditions [13]. In addition, in wt *Arabidopsis*, the activity of the antioxidant enzymes ascorbate peroxidase and catalase and the

Abbreviations: AGP, ADP-glucose pyrophosphorylase; chl, chlorophyll; FNR, ferredoxin-NADP$^+$ reductase; LD, long day; LHCI/II, light harvesting complex I/II; NDH, NADPH dehydrogenase; POBN, α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone; PRX, peroxiredoxin; PSI/II, photosystem I/II; ROS, reactive oxygen species; SD, short day; SOD, superoxide dismutase

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doi:10.1016/j.bbabio.2011.11.014

content of reduced glutathione was decreased in short day conditions [14]. A lower performance of the antioxidant system may result in a higher level of oxidative stress.

In the present study, we investigated whether changes in the length of the light period alter the properties of the photosynthetic electron transport chain and thereby change the amount of ROS generated in the light. A higher level of ROS in a mutant background may trigger the manifestation of a phenotype. To test this hypothesis we investigated ROS generation in leaves and isolated thylakoids from tobacco and *Arabidopsis* grown at the same light intensity under a 8 h light/16 h dark (short day, SD) and 16 h light/ 8 h dark regime (long day, LD). We followed the light-induced generation of ROS by spin trapping EPR spectroscopy in leaves and thylakoids. Furthermore, we investigated the susceptibility to light by measuring the loss of variable fluorescence in leaves. The electron transport capacities of isolated thylakoids, the capacities of formation of a proton gradient and the production of ATP were measured. We show that SD leaves and even isolated thylakoids from SD leaves produce significantly more superoxide by the pseudocyclic electron flow than those from leaves in LD conditions. We suggest that an alteration at photosystem I (PSI) is responsible for the increase in ROS production in SD conditions. We postulate that this alteration is caused by the binding of an unknown protein to PSI.

2. Materials and methods

2.1. Materials

Nicotiana tabacum cv. Petit Havanna plants were grown for 8 weeks in soil either under short day conditions (8 h continuous white light—160 μmol quanta $m^{-2} s^{-1}$, 21 °C/16 h dark, 18 °C) or long day conditions (16 h continuous white light—160 μmol quanta $m^{-2} s^{-1}$, 21 °C/8 h dark, 18 °C).

2.2. Extraction of thylakoids from N. tabacum

Young fully expanded leaves were grinded in 0.33 M sorbitol, 60 mM KCl, 10 mM EDTA, 1 mM $MgCl_2$, 25 mM Mes pH 6.1. After centrifugation, the pellet was first washed with 0.33 M sorbitol, 60 mM KCl, 10 mM EDTA, 1 mM $MgCl_2$, 25 mM HEPES pH 6.7, then resuspended in 5 mM $MgCl_2$, 20 mM K_2PO_4/$KHPO_4$ pH 7.6 to break all intact chloroplast. After centrifugation, the pellet was resuspended in 0.3 M sucrose, 5 mM $MgCl_2$, 20 mM K_2PO_4/$KHPO_4$ pH 7.6 (measurement buffer) so as to have a final concentration of chlorophyll of ca. 1 mg of chlorophyll per ml of thylakoids. All centrifugations were performed at 3000*g* for 3 min at 4 °C.

2.3. Room-temperature spin-trapping EPR measurements

Spin-trapping assays with 4-pyridyl-1-oxide-*N*-*tert*-butylnitrone (4-POBN) (Sigma-Aldrich) were carried out using leaf disks or freshly shocked chloroplasts at a concentration of 10 μg of Chl ml^{-1}. Leaf disks were vacuum-infiltrated with the buffer containing the spin trap reagents prior to the illumination and then floating on the same buffer during the illumination. Samples were illuminated for a given time with white light (500 or 1500 μmol quanta $m^{-2} s^{-1}$) in the presence of 50 mM 4-POBN, 4% ethanol, 50 μM Fe-EDTA, and buffer (25 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 0.3 M sorbitol). When required, 20 μM DCMU, 50 μM DNP-INT or 10 μM DBMIB, or 10 mM NH_4Cl were added prior illumination. Alternatively, 5 μM nigericin was used as uncoupler.

Spin-trapping assays with 5-diethoxyphosphoryl-5-methyl-1-pyrroline N-oxide (DEPMPO) were carried out with thoroughly washed thylakoids (washing buffer contained 25 mM HEPES, pH 7.5, and 5 mM $MgCl_2$) at a concentration of 50 μg of chl ml^{-1}. Samples were illuminated for 5 min with white light (1500 μmol quanta $m^{-2} s^{-1}$) in the presence of 50 mM DEPMPO, 1 mM DTPA and buffer (25 mM HEPES, pH 7.5, 5 mM $MgCl_2$). When required, 50 μg SOD/ml, or 10 μM DBMIB were added prior to the illumination.

EPR spectra were recorded at room temperature in a standard quartz flat cell using an ESP-300 X-band (9.73 GHz) spectrometer (Bruker, Rheinstetten, Germany). The following parameters were used: microwave frequency 9.73 GHz, modulation frequency 100 kHz, modulation amplitude: 1 G, microwave power: 63 mW in DEPMPO assays, or 6.3 mW in 4-POBN assays, receiver gain: 2×10^4, time constant: 40.96 ms; number of scans: 4.

2.4. O_2 measurements

Measurements of O_2 production and consumption were performed in a Liquid-Phase Oxygen Electrode Chamber (Hansatech Instruments, Norfolk, England). O_2 evolution was measured in thylakoids (10 μg Chl ml^{-1}) in the presence of 1 mM ferricyanide, O_2 consumption was measured in thylakoids (40 μg Chl ml^{-1}) in the measurement buffer in the presence or absence of 10 mM NH_4Cl. PSI activity was measured from thylakoids (5 μg Chl ml^{-1}) in the presence of 10 μM DCMU, 5 mM ascorbate, 30 μM DCPIP and 500 μM methylviologen. All activities were measured in the absence or presence of 10 mM NH_4Cl as an uncoupler. Alternatively, 5 μM nigericin was used as an uncoupler.

2.5. Pigment content

Leaf disks from young fully expanded leaves were grinded in liquid nitrogen and resuspended in 2 ml ethanol 98%. After 2 min centrifugation at room temperature and 10,000*g*, pigment content was determined spectrophotometrically from the optical densities measured at 470, 534, 643, 661 and 750 nm [15]. At least two leaf disks were analyzed for each plant considered.

2.6. Immunoblots

15 μg Chl from the different thylakoids extracts were used for analysis by SDS-PAGE (12% acrylamide) and immunoblotting. Proteins were blotted onto nitrocellulose filters. Labelling of the membranes with anti-Cytf, ATP synthase β-subunit (Agrisera, Vännäs, Sweden), PsaD, PsbA, FNR, NDH-H, or PRX-Q antisera was carried out at room temperature in 1× TBS (50 mM Tris–HCl pH 7.6, 150 mM NaCl), 0.1% Tween 20 and 1% non-fat powder milk. After washing, bound antibodies were revealed with a peroxidase-linked secondary anti-rabbit antibody (Agrisera, Vännäs, Sweden) and visualized by enhanced chemiluminescence.

2.7. Measurement of the proton gradient by 9-aminoacridine fluorescence

The light-induced generation of a proton gradient was measured in thylakoids (20 μg Chl ml^{-1}) in the presence of 7 μM 9-aminoacridine in 0.3 M sucrose, 5 mM $MgCl_2$, 20 mM K_2PO_4/$KHPO_4$ pH 7.6 using the NADPH measuring head of the DUAL-PAM (Walz, Effeltrich, Germany). As actinic light, red light at 500 μmol quanta $m^{-2} s^{-1}$ was used. 100 μM methylviologen was added as an electron acceptor when indicated.

2.8. ATP production

Thylakoids (0.5 mg Chl ml^{-1}) were illuminated for 2 min at 1500 μmol quanta $m^{-2} s^{-1}$ in 25 mM Tricine buffer pH 8.0 containing 5 mM K_2PO_4/$KHPO_4$ pH 8.0, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM ferricyanide, 5 mM glucose, 0.2 mM ADP and 2.5 U of hexokinase. After illumination, the reaction was stopped by addition of 0.3 M $HClO_4$. 1 ml of the sample was mixed with 1 M Tris (final Tris concentration 50 mM) and neutralized to pH 8.0 with 3 M KOH. After 15 min of incubation on ice, the neutralized sample was centrifuged for 5 min at

5000*g*. Determination of ATP production was then assayed following NADPH production with a spectrophotometer at OD 340 nm, using 500 µl of the supernatant mixed with an equal volume of 50 mM Tricine buffer pH 8.0, 0.5 mM $NADP^+$ and 5 mM $MgCl_2$. The reaction was started by adding glucose-6-phosphate dehydrogenase. A repetition of all samples was done in the presence of 50 mM NH_4Cl as an uncoupler to determine the intrinsic activity of the adenylate kinase.

2.9. Statistics

Data represent means or representative examples from measurements repeated three to nine times on independent preparations. Typical SD values are shown in Figs. 1, 3, 7, 9, 10 and Table 1.

3. Results

3.1. Influence of the length of the light period on the generation of reactive oxygen species in leaves

Long-day conditions compared to short-day conditions affected many aspects of growth and development of tobacco plants. Stem elongation was greatly enhanced by long photoperiods, the size of the leaves and the root system were largely increased, the leaves were thicker and appeared greener, and the plants started earlier to flower, indicative for a difference in developmental patterns (data not shown). In the present study we focus only on aspects related to photosynthetic electron transport.

It was reported previously that shortening of the photoperiod alters the photosynthetic structures in a way that resemble the acclimation to low light [16]. Plants acclimated to low light are more susceptible to photoinhibition than plants acclimated to high light [17] and higher photooxidative stress may explain why many mutations show a clear phenotype under SD conditions. To investigate whether the short-day grown plants were more susceptible to photoinhibition, leaves of SD and LD plants were first incubated for 4 h in the dark in lincomycin to block the synthesis of D1 protein and thereby the repair of damaged photosystem II (PSII) centers. Then leaves were illuminated for up to 2 h with 1500 µmol quanta $m^{-2}\,s^{-1}$ and the ratio of Fv/Fm, a measure of the maximum quantum yield of PSII, was determined (Fig. 1). Prior to the high light treatment, Fv/Fm was 0.82 for both leaves from SD and LD plants. This value is typically measured in higher plants [18]. During high light exposure, the loss of variable fluorescence was considerably higher in leaves from SD plants than in those from LD plants showing that SD leaves are more susceptible to photoinhibition. In the absence of lincomycin no significant difference between SD and LD plants was found (data not shown).

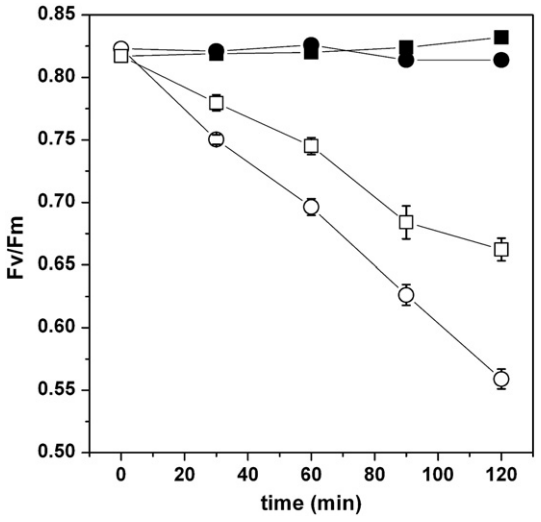

Fig. 1. Photoinhibition. Effect of high light on chlorophyll fluorescence in leaves from short day and long day grown plants. Leaf discs were floating on lincomycin solution. Circles, short day; squares, long day, filled symbols, incubated in low light (8 µmol quanta $m^{-2}\,s^{-1}$), open symbols incubated in high light (1500 µmol quanta $m^{-2}\,s^{-1}$). Error bars represent S.D. (four independent experiments on four plants).

Table 1
ROS production in SD and LD thylakoids. Thylakoids were illuminated for 1 min at high light (1500 µmol quanta $m^{-2}\,s^{-1}$) in the presence of the spin trap 4-POBN (50 mM)/ethanol. Where indicated 10 µM DCMU, or 10 mM NH_4Cl were added prior to the illumination. The double integral of the total signal obtained with SD wt thylakoids after illumination was set to unity. Error bars represent SD (four independent experiments for long day-grown plants, three for short day-grown plants and three for *N. sylvestris*).

	SD thylakoids	LD thylakoids
N. tabacum		
no addition	1	0.53 ± 0.07
+ 10 µM DCMU	0.20 ± 0.04	0.20 ± 0.01
+ 10 mM NH_4Cl	1.76 ± 0.05	1.71 ± 0.01
N. sylvestris variety		
no addition	1	0.59 ± 0.06

Photoinhibition is often caused by light-induced production of reactive oxygen species. The production of $^{\bullet}OH$ derived from H_2O_2 in the presence of Fe(II) can be demonstrated by electron paramagnetic resonance (EPR) spectroscopy using ethanol/α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN) as a specific spin-trapping system [19,20]. During photosynthetic electron transport the reduction of O_2 to $O_2^{\bullet-}$ may occur under conditions of limited number of electron acceptors other than O_2. Two molecules of $O_2^{\bullet-}$ dismutate, either spontaneously or catalyzed by superoxide dismutase (SOD), to H_2O_2 and O_2. In the presence of reduced transition metal ions such as Fe^{2+}, H_2O_2 can give rise to the hydroxyl radical ($^{\bullet}OH$) in a Fenton reaction supported by the reduction of Fe^{3+} by $O_2^{\bullet-}$ [21]:

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+},$$

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2.$$

The combination of these two reactions is known as the Fe-catalyzed Haber–Weiss reaction:

$$H_2O_2 + O_2^{\bullet-} \xrightarrow{Fe^{2+}/Fe^{3+}} {}^{\bullet}OH + OH^- + O_2.$$

In the present study, leaf disks of short-day and long-day tobacco plants were illuminated with a high light intensity of white light (1500 µmol quanta $m^{-2}\,s^{-1}$) in the presence of POBN/ethanol/FeEDTA. Representative spectra are shown in Fig. 2. A significantly larger EPR signal was obtained from leaf disks of SD plants than of LD plants after 60 min light treatment. Taken this result together with the higher loss in Fv/Fm in SD leaves, this demonstrates the involvement of ROS in photodamage of PSII and not only in the repair of PSII (for an actual debate on the mechanisms of photoinhibition see [22]).

Next we investigated whether the high yield of ROS production and the high susceptibility of SD leaves to photoinhibition were caused by a difference in pigment composition (Fig. 3). Leaves of short-day plants are thinner and about 20% lighter than those of long-day plants (data not shown). Neither the chlorophyll a, b, carotenoid and anthocyanins contents nor the chl a/b ratio were significantly altered in SD leaves compared to LD leaves when the pigment content was normalized to the fresh weight. This excludes the possibility that the higher susceptibility to photoinhibition of SD plants was caused by differences in the pigment composition or antenna size. However, one has to keep in mind that SD leaves are

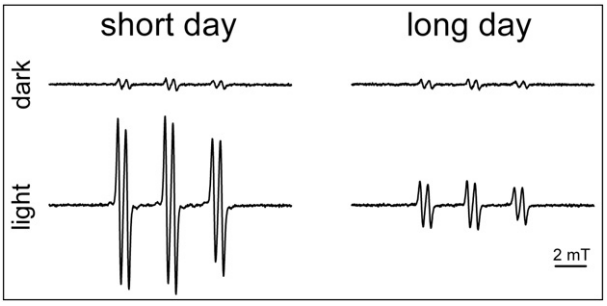

Fig. 2. Light-induced hydroxyl radical formation in SD and LD leaf disks. Generation of hydroxyl radicals is shown by indirect spin trapping with 4-POBN. Typical EPR spectra of the 4-POBN/α-hydroxyethyl adduct are shown. After infiltration with 1 ml of 4-POBN/ethanol/FeEDTA mix, leaf disks were incubated in the same medium for 1 h in the dark or under high light (1500 μmol quanta $m^{-2} s^{-1}$) before detection of the radicals in the medium.

thinner and light may penetrate better into the leaves and may therefore evoke larger damage.

3.2. Influence of the length of the light period on the generation of reactive oxygen species in thylakoids

The question arose whether the increased light-induced ROS production in SD leaves was caused by a less efficient antioxidant system or a difference in the metabolic state of the SD leaves compared to LD leaves or whether it was due to differences in the photosynthetic electron transport. To address the latter point, we isolated thylakoid membranes and washed them thoroughly. Washing of thylakoids in 5 mM $MgCl_2$ removes the chloroplastic SOD and soluble ascorbate peroxidase. The same spin trapping assay for the detection of $^{\bullet}OH$ production as in Fig. 2 was repeated with thylakoids (Fig. 4). In absence of FeEDTA, no signal is detected, meaning that no $^{\bullet}OH$ is directly produced by illuminated thylakoids. Thylakoids from SD leaves produced twice as much ROS as those from LD leaves in the absence of an uncoupler. Addition of DCMU, an inhibitor of the electron transport in PSII, almost completely inhibited spin adduct formation in both samples. Addition of an uncoupler (NH_4Cl or nigericin were used) lead to an increase of the formation of the spin adduct and abolished the difference between the two samples. The increase in ROS production can be explained by a release of the limitation of electron transport at low luminal pH at the level of the cytochrome b_6f complex [23]. A quantitative comparison of the sizes of the EPR signals is given in Table 1. This effect was seen in fully expanded leaves. When very old leaves or very young leaves were used instead, the differences between thylakoids from SD and LD plants were much smaller or even not visible. To show that not only thylakoids from tobacco but also from a different species behave the same way, we compared thylakoids from SD and LD *Arabidopsis thaliana* plants. Thylakoids from *A. thaliana* grown under SD generated also twice the amount of ROS compared to thylakoids from plants grown under LD (Fig. S1, supplementary material). In addition, we used a variety of *N. sylvestris* that has lost the difference in flowering induction due to the length of the photoperiod. When these plants were grown under SD photoperiod they produced twice as much ROS compared to those grown in LD photoperiod (Table 1). This indicates that differences in the developmental state of *N. tabacum* grown in SD and LD conditions did not contribute to the phenomenon observed here.

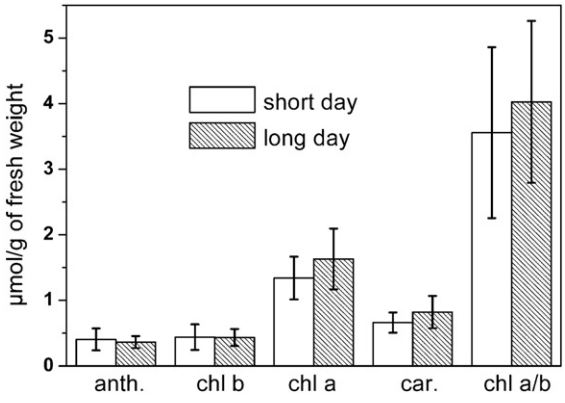

Fig. 3. Pigment content in SD and LD leaves. Anthocyanins, chlorophyll a and b, carotenoids content and chlorophyll a/b ratio were determined spectrophotometrically after ethanol extraction of the pigments from leaf disks from seven plants. Pigment content is reported as mean ± SD.

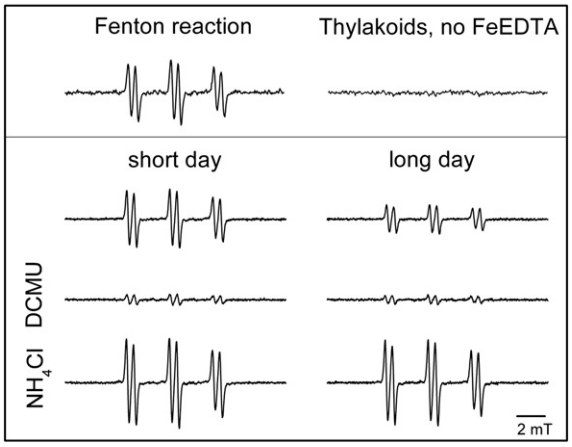

Fig. 4. Light-induced hydroxyl radical formation in SD and LD thylakoids. Generation of hydroxyl radicals is shown by indirect spin trapping with 4-POBN/ethanol/FeEDTA. Upper part: EPR spectrum of the 4-POBN/α-hydroxyethyl adduct produced by the Fenton reaction in the presence of 5 μM H_2O_2 (left), and EPR spectrum obtained with illuminated thylakoids in the absence of FeEDTA. Lower part: samples were illuminated for 1 min with 1500 μmol quanta $m^{-2} s^{-1}$. Where indicated, 20 μM DCMU, 50 μM DNP-INT, or 10 mM NH_4Cl were added prior illumination. Typical EPR spectra of the 4-POBN/α-hydroxyethyl adduct are shown.

To investigate whether $O_2^{\bullet-}$ was the primarily radical species formed, experiments were carried out in the presence of DEPMPO instead of POBN (Fig. 5). In the spin-trapping assay with POBN, $^{\bullet}OH$ is trapped in a metal-ion assisted Fenton mechanism from H_2O_2, the disproportionation product of $O_2^{\bullet-}$. DEPMPO forms specific and distinguishable adducts with $O_2^{\bullet-}$ and $^{\bullet}OH$ [24]. The spin adduct formed

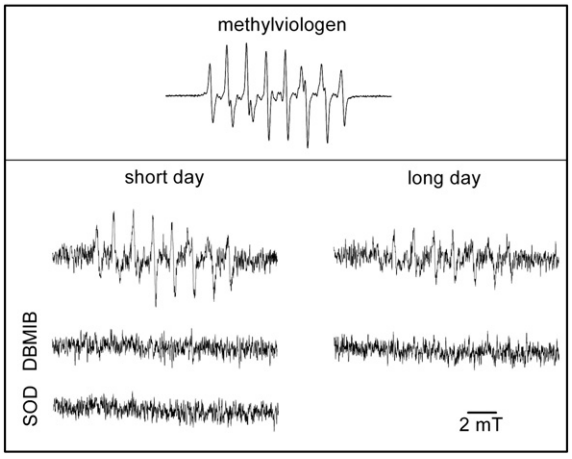

Fig. 5. Light-induced superoxide formation in SD and LD thylakoids. Upper part: EPR spectrum of the DEPMPO-OOH adduct generated in thylakoids in the light in the presence of 10 μM methylviologen. Lower part: thylakoid samples were illuminated for 2 min with 500 μmol quanta $m^{-2} s^{-1}$ in the presence of 50 mM DEPMPO. Where indicated, 50 μg SOD/ml, or 10 μM DBMIB was added prior to the illumination. Typical EPR spectra of the DEPMPO-OOH adduct are shown.

after illumination of SD and LD thylakoids can be attributed to DEMPO/$O_2^{\bullet-}$ (DEPMPO-OOH; the asymmetrical signal of which could be simulated by a 1 :1 mixture of the two species having the coupling constants : $a_N = 13.1$ (13.2); $a_P = 50.7$ (49.9) and $a_{H\beta} = 11.7$ (10.5) G). In the presence of SOD, the signals were completely suppressed. SD thylakoids produced a signal which was 1.8 fold larger than the signal obtained in LD thylakoids. When the cytochrome b_6f inhibitor DBMIB was added prior to the illumination, no spin adducts could be detected. This demonstrates that the $O_2^{\bullet-}$ is generated at the level of photosystem I (PSI) by the so-called pseudocyclic electron flow, also known as Mehler reaction. A production of $O_2^{\bullet-}$ at the level of PSII [25] or via the plastid terminal oxidase [20] can thereby be excluded in the samples and experimental conditions used here.

Furthermore, we investigated whether the amount of light-generated $^1O_2$ depends also on the growth conditions by using the spin trap TEMPD, which reacts specifically with $^1O_2$ [26]. No difference in the two types of samples, SD and LD thylakoids from tobacco and from *Arabidopsis*, was observed (Fig. S2, Supplementary figure).

To see whether the length of the day alters the amount of proteins involved in photosynthetic electron transfer, we monitored the level of 6 key thylakoid membrane proteins by immunoblot: PsaD, a core protein of PS I, D1, a core protein of PS II, cytochrome f, NDH-H, a protein of the NDH complex, FNR and the β-subunit of the ATP synthase. In addition, we probed for peroxiredoxin Q, an antioxidant enzyme which is bound to the thylakoid membrane and possibly active under our experimental conditions [27]. Fig. 6 shows that the level of the tested proteins was unchanged in SD and LD thylakoids.

Next the light dependency of the O_2-consumption of SD and LD thylakoids was measured using a Clark-type oxygen electrode (Fig. 7A). In the absence of an uncoupler, SD thylakoids consumed twice as much oxygen as did LD thylakoids. In the presence of an uncoupler, no significant difference between the two samples was observed. These results are in agreement with the spin trapping experiments: Thylakoids from short-day plants produce more $O_2^{\bullet-}$ in a coupled state, while the maximum capacity of the electron transport is the same as seen in the presence of an uncoupler. In this experimental setup, the electron donor is water and O_2 is evolved at PSII while it is consumed at the acceptor side of PSI. The higher O_2-consumption in SD thylakoids was also observed when $DCPIPH_2$ was added as artificial donor to PSI and the activity of PSII was inhibited by the addition of DCMU. In this case, at saturating light intensity, SD thylakoids consumed 8.7 ± 0.7 μmol O_2 mg Chl^{-1} min^{-1}, LD thylakoids 6.5 ± 0.3 μmol O_2 mg Chl^{-1} min^{-1} and, in the presence of nigericin, both type of samples produced 19.4 ± 1.3 μmol O_2 mg Chl^{-1} min^{-1}. This result shows that the difference between SD and LD thylakoids is localized at PSI when a proton gradient is present.

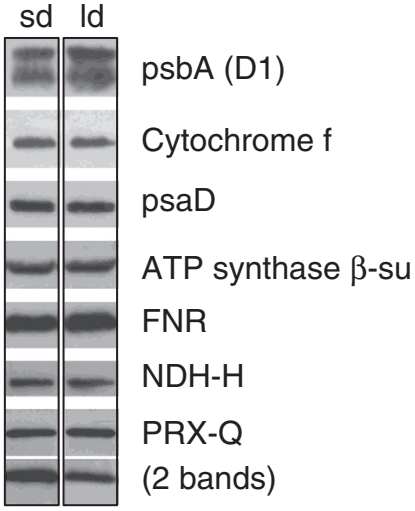

Fig. 6. Protein content in SD and LD thylakoids. Protein composition of the thylakoid extracts from short day or long day grown plants was analyzed by SDS-PAGE and immunoblotting with antisera against PsaD (photosystem I), PsbA (photosystem II), Cytf (cytochrome b6/f), ATP synthase β-subunit, ferredoxin-$NADP^+$ reductase (FNR), NADPH dehydrogenase (NDH) H-subunit, or peroxiredoxin (PRX) Q.

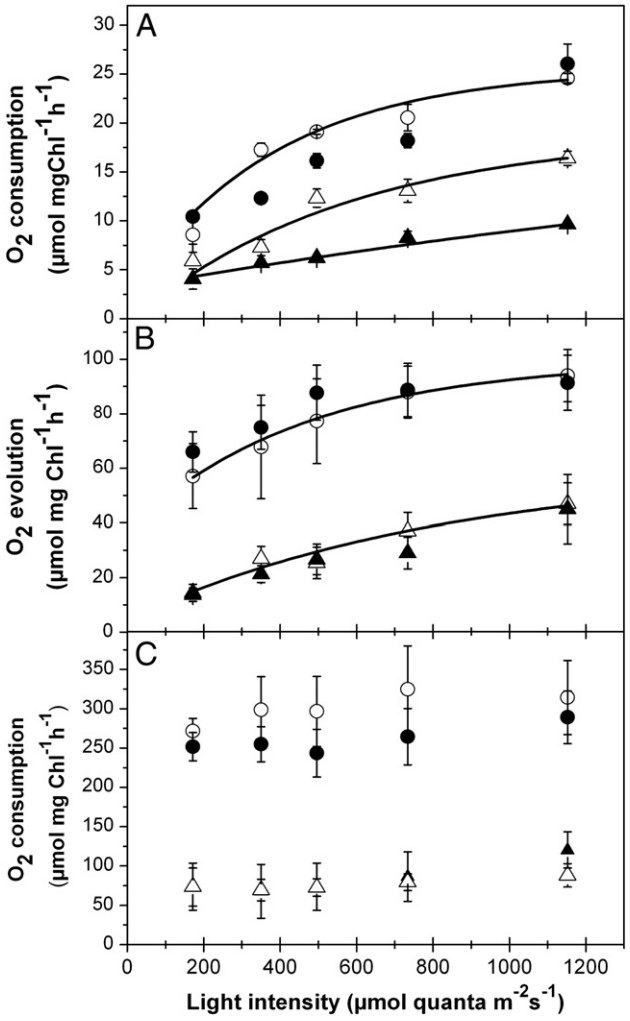

Fig. 7. Electron transport activities of SD and LD thylakoids. A. O_2 consumption in short day and long day plants. O_2 consumption by thylakoids from short day (open symbols) or long day –grown (filled symbols) plants was measured under different light intensities in the presence (circles) or absence (triangles) of 10 mM NH_4Cl as an uncoupler. O_2 consumption is reported as mean ± SD of 4 experiments. B. O_2 evolution in short day and long day plants. O_2 production by thylakoids from short day (open symbols) or long day—grown (filled symbols) plants were measured under different light intensities in the presence of 1 mM ferricyanide, with (circles) or without (triangles) 10 mM NH_4Cl as an uncoupler. O_2 evolution is reported as mean ± SD of 4 experiments. C. PSI activity in short day and long day plants. PSI activity by thylakoids from short day (open symbols) or long day—grown (filled symbols) plants were measured under different light intensities in the presence of 10 μM DCMU, 5 mM ascorbate, 30 μM DCPIP and 500 μM methylviologen, with (circles) or without (triangles) 10 mM NH_4Cl as an uncoupler. PSI activity is reported as mean ± SD of three experiments.

Fig. 7B shows the light dependency of the linear electron transport measured from water to $K_3[Fe(CN)_6]$, and Fig. 7C the PSI electron transport measured from $DCPIPH_2$ to methylviologen in the presence of DCMU. In the absence of an uncoupler, $DCPIPH_2$ is a very inefficient electron donor because the low pH in the lumen increases the midpoint potential of the DCPIP/$DCPIPH_2$ redox couple (Em = 217 mV at pH 6.5). No difference between SD and LD thylakoids was seen neither in the activity of linear electron flow in the presence of an artificial electron acceptor nor on PSI electron transport activity.

The higher electron transport activity in SD thylakoids in the absence of an electron acceptor other than oxygen resulted in the formation of an almost 2-fold larger proton gradient as shown by measuring the quenching of 9-aminoacridine fluorescence (Fig. 8). The quenching of the 9-aminoacridine fluorescence was rather small in the absence of an electron acceptor other than O_2. Charge recombination reaction in PSI in the absence of an artificial electron acceptor may be responsible for the small proton gradient. Down-regulation of electron transport by photosynthetic control at the b_6f complex cannot be the only explanation because in this case the proton gradient should be as large as in the presence of an artificial acceptor. When methylviologen was added as electron acceptor, the quenching of 9-aminoacridine fluorescence was strongly increased in both samples. It was still a bit larger in SD thylakoids than in LD thylakoids. The slightly larger proton gradient resulted in a slightly higher ATP-production when thylakoids were illuminated in the presence of $K_3[Fe(CN)_6]$ as artificial electron acceptor (Fig. 9). Even in the absence of an artificial electron acceptor, a small amount of ATP was produced in SD thylakoids while no ATP was produced in LD thylakoids (data not shown). This shows that pseudocyclic electron flow can be sufficient to drive ATP-synthesis at a low level in SD thylakoids.

Taken together, the presented results point towards a modification at the level of PSI which allows PSI in SD thylakoids to use O_2 more efficiently as electron acceptor than in LD thylakoids. This difference is eliminated in the presence of the uncouplers NH_4Cl or nigericin. By monitoring the ROS level by POBN we tested whether reductants like DTT, reduced glutathione, NADH or NADPH induced modifications in PSI which could explain the differences in O_2 reduction and concomitant ROS production. NADPH was the only substance that affected the ROS production differently in SD and LD thylakoids. As shown in Fig. 10, NADPH doubled the ROS production in LD thylakoids and suppressed thereby the difference between SD and LD thylakoids in the coupled state. This was not the case when NADH was added.

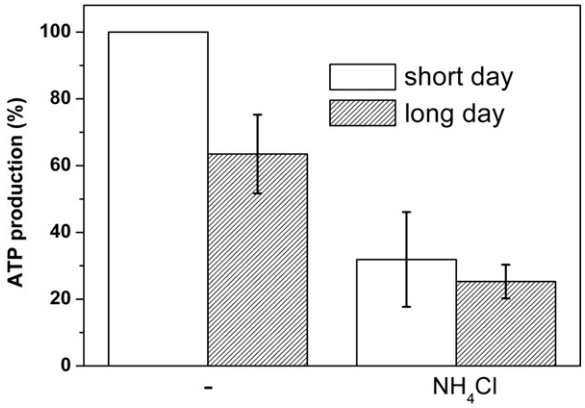

Fig. 9. ATP production in short day and long day plants. The accumulation of ATP in thylakoids from short day or long day-grown plants, illuminated for 2 min at 500 μmol quanta m^{-2} s^{-1}, in the presence of 1 mM $K_3[Fe(CN)_6]$ and in absence or the presence of 50 mM NH_4Cl, was measured indirectly by determination of NADPH synthesis in the presence of hexokinase and glucose-6-phosphate dehydrogenase. ATP production is given as percentage and reported as mean ± SD of four experiments.

4. Discussion

All three modes of photosynthetic electron transport—linear, cyclic and pseudocyclic—are likely to be at work *in vivo* [28]. It seems just that the proportions in favour of pseudocyclic electron flow are changed in SD compared to LD thylakoids. As shown in Figs. 4, 5, 7, the O_2 consumption/$O_2^{\bullet-}$ production is higher in SD than in LD thylakoids. Pseudocyclic electron transport, like cyclic electron transport, contributes to the formation of the proton gradient and to the production of extra ATP without reducing $NADP^+$ (Figs. 8, 9). An increase in the proportion of pseudocyclic electron flow as well as in cyclic electron flow may explain the higher O_2 consumption/$O_2^{\bullet-}$ production in SD thylakoids under the assumption that some electrons can leak out to O_2 during cyclic flow. Two routes for cyclic electron flow are discussed in the literature [29,30]: one depends on the NDH complex, the other one on the formation of a supercomplex between PSI-LHCI-LHCII-FNR-Cytb_6f-PGRL1 [29,31]. The pathway of cyclic electron flow that involves electron transport via the NDH complex is found to be increased in wt plants under water stress [32], in mutants of the glyceraldehyde-3-phosphate dehydrogenase [33], and in mutants of the chloroplast

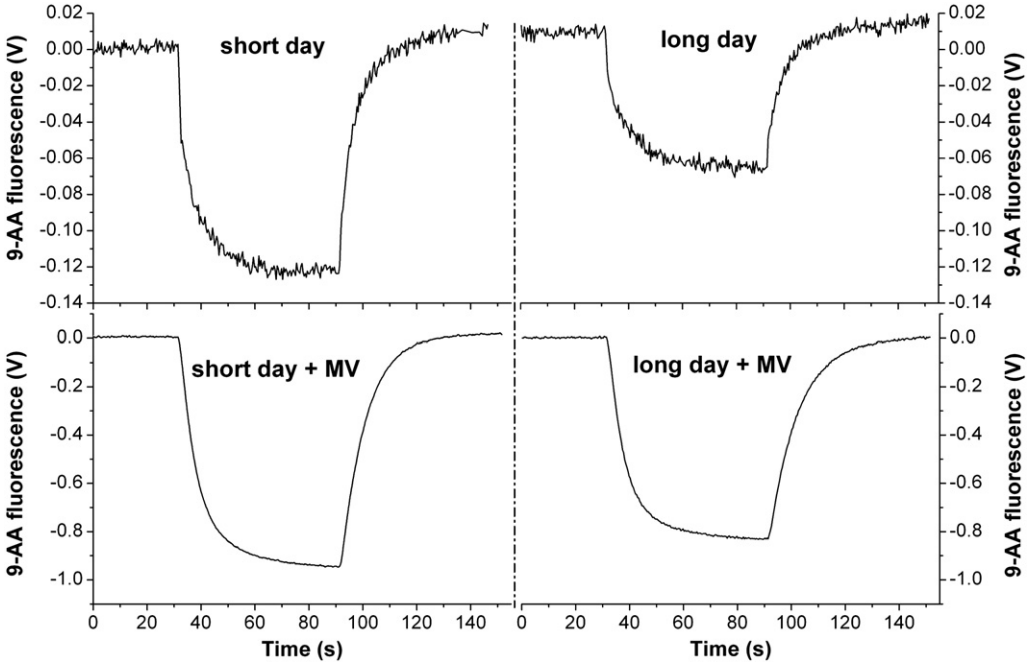

Fig. 8. 9-aminoacridine fluorescence in SD and LD thylakoids. 7 μM 9-aminoacridine was added to the thylakoid membranes. Actinic red light (500 μmol quanta m^{-2} s^{-1}) was switched on after 30 s and switched off after 90 s. When indicated, 100 μM methylviologen was added prior to the measurement.

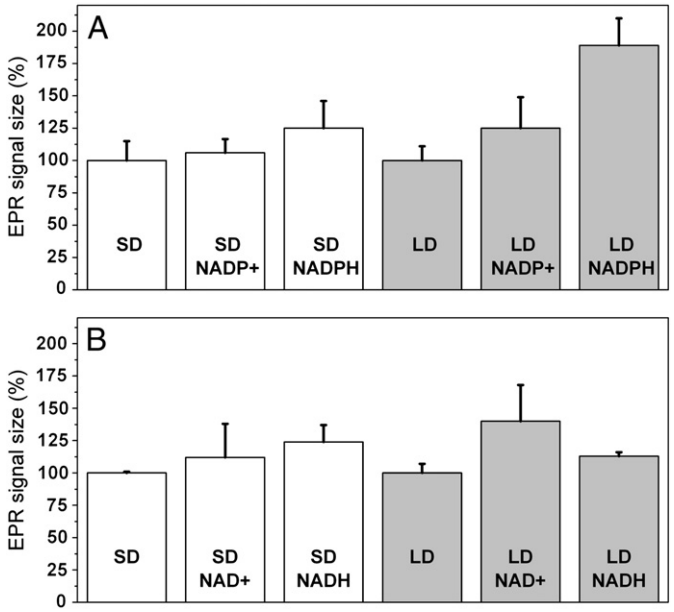

Fig. 10. Effect of NAD(P)H on the light-induced hydroxyl radical formation in SD and LD thylakoids. Average amplitude of EPR spectra obtained for thylakoid samples illuminated for 1 min at high light (1500 μmol quanta m^{-2} s^{-1}). The signal size obtained without addition of NAD(P)H was set to 100%. Error bars represent SD (seven independent experiments for long day-grown plants and six for short day-grown plants).

fructose-1,6-bisphosphatase [34]. In these plants, the protein level of NDH is increased. As shown in Fig. 6, the content of the NDH complex is unchanged in SD conditions and it seems therefore to be unlikely that this pathway plays a role in the stimulation of $O_2^{\bullet-}$ generation in SD conditions. The contribution of cyclic electron flow via the formation of a supercomplex to the phenomenon observed in SD thylakoids can also be ruled out based on the fact that the level of FNR was unchanged (Fig. 6) and that ferredoxin was lost in the washed thylakoids. No traces of ferredoxin were detected in immunoblots and by low temperature EPR spectroscopy. In addition, the pgr5 mutant of *Arabidopsis* showed the same increase in $O_2^{\bullet-}$ generation in SD conditions as the wt (Supplementary Fig. 3). Alternatively, a decrease of the amount of FNR could increase the reduction of O_2 at PSI, as has been shown to occur in transgenic tobacco with reduced level of FNR [35], but this is not the case here since the level of FNR was the same in both types of samples.

The difference in O_2 consumption between SD and LD thylakoids was maintained when $DCPIPH_2$ was used as electron donor to PSI and, furthermore, $O_2^{\bullet-}$ production was enhanced in LD thylakoids by the addition of NADPH (Fig. 10). These observations point to a change of the properties of the acceptor side of PSI which facilitates the reduction of O_2.

There exits several possibilities to rationalize these observations: (i) A higher diaphorase activity of FNR in SD than in LD thylakoids.[1] The diaphorase activity measured in SD and LD thylakoids was identical (data not shown), therefore we exclude this possibility to play a role under the experimental conditions used here. (ii) Redox poising by NADPH which allows more efficient reduction of O_2. Redox poising was suggested to regulate cyclic electron flow [36,37]. If the redox potential played a role in the regulation of pseudocyclic electron flow, NADH or reductants like DTT or reduced glutathione should induce the same effect like NADPH. Since this was not the case, we exclude this possibility. (iii) NADPH-dependent reversible conformational changes of PSI that facilitate the reduction of O_2. Rajagopal et al. [38] observed a quenching of the 77 K chlorophyll fluorescence upon the addition of $NADP^+$ which was reversed by NADPH, and they proposed that a conformational modification of FNR upon binding of $NADP^+$ or NADPH triggers conformational changes of the PSI protein complex. Under our condition we would have to assume that a higher concentration of NADPH is still present in thoroughly washed thylakoids, which seems unlikely to occur. Furthermore, we were unable to reproduce the reversible $NADP^+$-induced quenching of chlorophyll fluorescence, neither in SD nor in LD thylakoids. However, one can imagine that, in addition, a putative protein plays a role in regulating pseudocyclic electron flow. We propose that this putative protein binds to PSI under SD conditions and that this protein facilitates the access of O_2 to its site of $O_2^{\bullet-}$ generation at the acceptor side of PSI. This protein should be able to sense the pH in the stroma or in the lumen, and, in the presence of an uncoupler, it could change its conformation and thereby of the access of O_2 to PSI. The differences between SD and LD thylakoids are abolished in the presence of an uncoupler. We propose further that this putative protein is localized close to the FNR binding site in SD plants and is lacking in LD plants.

In leaf disks a larger difference in the amount of light-induced ROS production was found between SD and LD plants than in isolated thylakoids (Figs. 2, 4). In SD leaves the ratio of $NADP^+$/ NADPH may be altered so that little $NADP^+$ is available as electron acceptor and that therefore the reduction of O_2 is favoured. In addition, it is known that the antioxidant system is affected by SD conditions. In *Arabidopsis*, it has been shown that the activity of the antioxidant enzymes ascorbate peroxidase and catalase and the content of reduced glutathione is decreased in short day conditions [14]. A higher activity of pseudocyclic electron flow and, in addition, a lower performance of the antioxidant system result in a higher level of oxidative stress. In addition, the FNR content and differences in the distribution of FNR isoforms [39,40] may contribute to oxidative stress *in vivo*. It has been demonstrated that plants expressing antisense FNR show increased susceptibility to photodamage [35,41] while FNR overexpressors show no difference in CO_2 assimilation but an enhanced tolerance to photooxidative stress [42].

As already mentioned in Introduction, starch synthesis is stimulated in SD plants [7]. Starch synthesis may be regarded as an overflow product when photosynthesis is high [43,44]. When the rate of photosynthesis is high relative compared to the demand of sucrose, the ratio of 3-phosphoglycerate to Pi will be high. This activates the ADP-glucose pyrophosphorylase (AGP) and thereby allows a higher partitioning of photosynthates towards starch than towards sucrose synthesis. Besides this allosteric control, post-translational regulation mechanisms of AGP have been described [7]. In short days the activity of ADP-glucose pyrophosphorylase is increased by post-translational regulation. In parallel, the amount of the sucrose-phosphate synthase is decreased. This leads to an increase of the ratio ADP-glucose pyrophosphorylase activity to sucrose-phosphate synthase activity which results in an increased partitioning to starch during the first part of the light period in short day conditions [7]. The regulation of the electron flow at the acceptor side of PSI and the increase in pseudocyclic electron flow may be of physiological importance to fulfill the higher requirement of ATP under SD conditions. The higher pseudocylic electron flow leads to higher level of ROS which may act as retrograde plastid signal that induces changes in the transcriptome. A higher level of photooxidative stress may result in more pronounced phenotypes of certain mutants under SD conditions.

Supplementary materials related to this article can be found online at doi:10.1016/j.bbabio.2011.11.014.

Acknowledgements

We would like to thank Eevi Rintamäki (University of Turku), Diana Kirilovsky, Pierre Sétif, Thomas Roach, and Bill Rutherford (all CEA Saclay) for scientific discussions and Véronique Mary (CEA

[1] A diaphorase is an enzyme which uses NAD(P)H as a substrate and reduces an artificial electron acceptor.

Saclay) for excellent technical assistance. DEPMPO was kindly provided by S. Pietri, and the spectra were simulated by M. Culcasi (both CNRS UMR 6264, Marseille). Antibodies directed against PsaD and PsbA were kindly provided by J.-D. Rochaix (University of Geneva), against peroxiredoxin Q by K.J. Dietz (University of Bielefeld), against FNR by W. Oettmeier (University of Bochum) and the antibody directed against NDH-H by D. Rumeau (CEA Cadarache). Seeds of *Arabidopsis* lacking PGR5 were kindly provided by T. Shikanai (University of Kyoto). This work was supported by the Agence Nationale de Recherche reference ANR-09-BLAN-0005-01.

References

[1] J. Sachs, Vorlesungen über Pflanzenphysiologie, Ed. Engelmann, Leipzig, Germany, 1887.
[2] M. Stitt, P.V. Bulpin, T. ap Rees, Pathway of starch breakdown in photosynthetic tissues of *Pisum sativum*, Biochim. Biophys. Acta 544 (1978) 200–214.
[3] N.J. Chatterton, J.E. Silvius, Photosynthate partitioning into starch in soybean leaves: I. Effects of photoperiod versus photosynthetic period duration, Plant Physiol. 64 (1979) 749–753.
[4] S.C. Huber, T.W. Rufty, P.S. Kerr, Effect of photoperiod on photosynthate partitioning and diurnal rhythms in sucrose phosphate synthase activity in leaves of soybean (Glycine max L. [Merr.]) and tobacco (*Nicotiana tabacum* L.), Plant Physiol. 75 (1984) 1080–1084.
[5] P. Matt, U. Schurr, D. Klein, A. Krapp, M. Stitt, Growth of tobacco in short-day conditions leads to high starch, low sugars, altered diurnal changes in the Nia transcript and low nitrate reductase activity, and inhibition of amino acid synthesis, Planta 207 (1998) 27–41.
[6] H.M. Easlon, J.H. Richards, Photosynthesis affects following night leaf conductance in *Vicia faba*, Plant Cell Environ. 32 (2009) 58–63.
[7] Y. Gibon, O.E. Blasing, N. Palacios-Rojas, D. Pankovic, J.H. Hendriks, J. Fisahn, M. Hohne, M. Gunther, M. Stitt, Adjustment of diurnal starch turnover to short days: depletion of sugar during the night leads to a temporary inhibition of carbohydrate utilization, accumulation of sugars and post-translational activation of ADP-glucose pyrophosphorylase in the following light period, Plant J. 39 (2004) 847–862.
[8] O. Thimm, O. Blasing, Y. Gibon, A. Nagel, S. Meyer, P. Kruger, J. Selbig, L.A. Muller, S.Y. Rhee, M. Stitt, MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes, Plant J. 37 (2004) 914–939.
[9] T. Reinhold, A. Alawady, B. Grimm, K.C. Beran, P. Jahns, U. Conrath, J. Bauer, J. Reiser, M. Melzer, W. Jeblick, H.E. Neuhaus, Limitation of nocturnal import of ATP into *Arabidopsis* chloroplasts leads to photooxidative damage, Plant J. 50 (2007) 293–304.
[10] M. Gibala, M. Kicia, W. Sakamoto, E.M. Gola, J. Kubrakiewicz, E. Smakowska, H. Janska, The lack of mitochondrial AtFtsH4 protease alters *Arabidopsis* leaf morphology at the late stage of rosette development under short-day photoperiod, Plant J. 59 (2009) 685–699.
[11] A. Lepisto, S. Kangasjarvi, E.M. Luomala, G. Brader, N. Sipari, M. Keranen, M. Keinanen, E. Rintamaki, Chloroplast NADPH-thioredoxin reductase interacts with photoperiodic development in *Arabidopsis*, Plant Physiol. 149 (2009) 1261–1276.
[12] U. Bechtold, D.J. Murphy, P.M. Mullineaux, *Arabidopsis* peptide methionine sulfoxide reductase2 prevents cellular oxidative damage in long nights, Plant Cell 16 (2004) 908–919.
[13] G. Queval, E. Issakidis-Bourguet, F.A. Hoeberichts, M. Vandorpe, B. Gakiere, H. Vanacker, M. Miginiac-Maslow, F. Van Breusegem, G. Noctor, Conditional oxidative stress responses in the *Arabidopsis* photorespiratory mutant cat2 demonstrate that redox state is a key modulator of daylength-dependent gene expression, and define photoperiod as a crucial factor in the regulation of H2O2-induced cell death, Plant J. 52 (2007) 640–657.
[14] B. Becker, S. Holtgrefe, S. Jung, C. Wunrau, A. Kandlbinder, M. Baier, K.J. Dietz, J.E. Backhausen, R. Scheibe, Influence of the photoperiod on redox regulation and stress responses in *Arabidopsis thaliana* L. (Heynh.) plants under long- and short-day conditions, Planta 224 (2006) 380–393.
[15] R.J. Porra, W.A. Thompson, P.E. Kriedemann, Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy, Biochim. Biophys. Acta 975 (1989) 384–394.
[16] A. Lepistö, E. Rintamäki, Coordination of plastid and light signalling pathways upon development of *Arabidopsis* leaves under various photoperiods, Molecular Plant (in press).
[17] C.B. Osmond, What is photoinhibition: some insights from comparisons of sun and shade plants, in: N.R. Baker, J.R. Bowyer (Eds.), Photoinhibition of photosynthesis: from molecular mechanisms to the field, BIOS Scientific Publishers, Oxford, 1994, pp. 95–110.
[18] O. Björkman, B. Demmig, Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins, Planta 170 (1987) 489–504.
[19] E. Janzen, Y. Wang, R. Shetty, Spin trapping with α-pyridyl 1-oxide N-tert-butyl nitrones in aqueous solutions. A unique electron spin resonance spectrum for the hydroxyl radical adduct. J. Am. Chem. Soc. 100 (1978) 2923–2925.
[20] E. Heyno, C.M. Gross, C. Laureau, M. Culcasi, S. Pietri, A. Krieger-Liszkay, Plastid alternative oxidase (PTOX) promotes oxidative stress when overexpressed in tobacco, J. Biol. Chem. 284 (2009) 31174–31180.
[21] B. Halliwell, J. Gutteridge, Free Radicals in Biology and Medicine Fourth Edition, Oxford University Press, UK, 2006.
[22] Special Issue: Photoinhibition, Physiol. Plant. 142 (2011) 1–104.
[23] C.H. Foyer, R. Furbank, J. Harbinson, P. Horton, The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves, Photosynth. Res. 25 (1990) 83–100.
[24] C. Frejaville, H. Karoui, B. Tuccio, F. Le Moigne, M. Culcasi, S. Pietri, R. Lauricella, P. Tordo, 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline N-oxide: a new efficient phosphorylated nitrone for the in vitro and in vivo spin trapping of oxygen-centered radicals, J. Med. Chem. 38 (1995) 258–265.
[25] P. Pospisil, Molecular mechanisms of production and scavenging of reactive oxygen species by photosystem II, Biochim. Biophys. Acta 1817 (2012) 218–231.
[26] E. Hideg, T. Kalai, K. Hideg, Direct detection of free radicals and reactive oxygen species in thylakoids, Methods Mol. Biol. 684 (2011) 187–200.
[27] P. Lamkemeyer, M. Laxa, V. Collin, W. Li, I. Finkemeier, M.A. Schottler, V. Holtkamp, V.B. Tognetti, E. Issakidis-Bourguet, A. Kandlbinder, E. Weis, M. Miginiac-Maslow, K.J. Dietz, Peroxiredoxin Q of *Arabidopsis thaliana* is attached to the thylakoids and functions in context of photosynthesis, Plant J. 45 (2006) 968–981.
[28] J.F. Allen, Cyclic, pseudocyclic and noncyclic photophosphorylation: new links in the chain, Trends Plant Sci. 8 (2003) 15–19.
[29] G. DalCorso, P. Pesaresi, S. Masiero, E. Aseeva, D. Schunemann, G. Finazzi, P. Joliot, R. Barbato, D. Leister, A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in *Arabidopsis*, Cell 132 (2008) 273–285.
[30] G.N. Johnson, Physiology of PSI cyclic electron transport in higher plants, Biochim. Biophys. Acta 1807 (2011) 384–389.
[31] M. Iwai, K. Takizawa, R. Tokutsu, A. Okamuro, Y. Takahashi, J. Minagawa, Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis, Nature 464 (2010) 1210–1213.
[32] K. Kohzuma, J.A. Cruz, K. Akashi, S. Hoshiyasu, Y.N. Munekage, A. Yokota, D.M. Kramer, The long-term responses of the photosynthetic proton circuit to drought, Plant Cell Environ. 32 (2009) 209–219.
[33] A.K. Livingston, A. Kanazawa, J.A. Cruz, D.M. Kramer, Regulation of cyclic electron flow in C plants: differential effects of limiting photosynthesis at ribulose-1,5-bisphosphate carboxylase/oxygenase and glyceraldehyde-3-phosphate dehydrogenase, Plant Cell Environ. 33 (2010) 1779–1788.
[34] A.K. Livingston, J.A. Cruz, K. Kohzuma, A. Dhingra, D.M. Kramer, An *Arabidopsis* mutant with high cyclic electron flow around photosystem I (hcef) involving the NADPH dehydrogenase complex, Plant Cell 22 (2010) 221–233.
[35] S. Hald, B. Nandha, P. Gallois, G.N. Johnson, Feedback regulation of photosynthetic electron transport by NADP(H) redox poise, Biochim. Biophys. Acta 1777 (2008) 433–440.
[36] G.N. Johnson, Thiol regulation of the thylakoid electron transport ChainA missing link in the regulation of photosynthesis? Biochemistry 42 (2003) 3040–3044.
[37] P. Joliot, G.N. Johnson, Regulation of cyclic and linear electron flow in higher plants, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13317–13322.
[38] S. Rajagopal, N.G. Bukhov, H.A. Tajmir-Riahi, R. Carpentier, Control of energy dissipation and photochemical activity in photosystem I by NADP-dependent reversible conformational changes, Biochemistry 42 (2003) 11839–11845.
[39] J. Grzyb, P. Malec, I. Rumak, M. Garstka, K. Strzalka, Two isoforms of ferredoxin: NADP(+) oxidoreductase from wheat leaves: purification and initial biochemical characterization, Photosynth. Res. 96 (2008) 99–112.
[40] A. Moolna, C.G. Bowsher, The physiological importance of photosynthetic ferredoxin NADP+ oxidoreductase (FNR) isoforms in wheat, J. Exp. Bot. 61 (2010) 2669–2681.
[41] J.F. Palatnik, V.B. Tognetti, H.O. Poli, R.E. Rodriguez, N. Blanco, M. Gattuso, M.R. Hajirezaei, U. Sonnewald, E.M. Valle, N. Carrillo, Transgenic tobacco plants expressing antisense ferredoxin-NADP(H) reductase transcripts display increased susceptibility to photo-oxidative damage, Plant J. 35 (2003) 332–341.
[42] R.E. Rodriguez, A. Lodeyro, H.O. Poli, M. Zurbriggen, M. Peisker, J.F. Palatnik, V.B. Tognetti, H. Tschiersch, M.R. Hajirezaei, E.M. Valle, N. Carrillo, Transgenic tobacco plants overexpressing chloroplastic ferredoxin-NADP(H) reductase display normal rates of photosynthesis and increased tolerance to oxidative stress, Plant Physiol. 143 (2007) 639–649.
[43] H. Eichelmann, A. Laisk, CO_2 uptake and electron transport rates in wild-type and a starchless mutant of *Nicotiana sylvestris* (the role and regulation of starch synthesis at saturating CO_2 concentrations), Plant Physiol. 106 (1994) 679–687.
[44] M. Stitt, Metabolic regulation of photosynthesis, Kluwer Academic Publishers, Dordrecht, 1996.