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State transitions revisited—a buffering system for dynamic low light acclimation of *Arabidopsis*

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Abstract The mobile part of the light-harvesting chlorophyll (chl) *a*/*b* protein complex (LHCII), composed of the Lhcb1 and Lhcb2 proteins, is the basic unit of chloroplast state transitions—the short term tuning system in balancing the excitation energy between Photosystem (PS) II and PSI. State transitions are catalysed by the thylakoid associated STN7 kinase, and we show here that besides the phosphorylation of the Lhcb1 and Lhcb2 proteins, also the phosphorylation of Lhcb4.2 (CP29) is under the control of the STN7 kinase. Upon growth of *Arabidopsis* WT and *stn7* mutant plants under low and moderate light conditions, the WT plants favoured state 2 whereas *stn7* was locked in state 1. The lack of the STN7 kinase and state transitions in *stn7* also modified the thylakoid protein contents upon long-term low light acclimation resulting, for example, in low Lhcb1 and in elevated Lhca1 and Lhca2 protein amounts as compared to WT. Adjustments of thylakoid protein contents probably occurred at post-transcriptional level since the DNA microarray experiments from each growth condition did not reveal any significant differences between *stn7* and WT transcriptomes. The resulting high Lhcb2/Lhcb1 ratio in *stn7* upon growth at low light was accompanied by lower capacity for NPQ than in WT. On the contrary, higher amounts of PsbS in *stn7* under moderate and high light growth conditions resulted in higher NPQ compared to WT and consequently also in a protection of PSII against photoinhibition. STN7 kinase and the state transitions are suggested to have a physiological significance for dynamic acclimation to low but fluctuating growth light conditions. They are shown to function as a buffering system upon short high light illumination peaks by shifting the thylakoids from state 2 to state 1 and thereby down regulating the induction of stress-responsive genes, a likely result from transient over-reduction of PSI acceptors.

Keywords *Arabidopsis* · Light acclimation · STN7 kinase · Thylakoid protein phosphorylation

Abbreviations

chl	chlorophyll
F_v/F_m	photochemical efficiency of PSII
HSP	heat shock protein
LHCII	light harvesting chlorophyll *a*/*b* protein complex
NPQ	non-photochemical energy quenching
PSI	photosystem I
PSII	photosystem II

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Introduction

Light harvesting antenna complexes absorb sunlight and transfer the excitation energy to PSII and PSI complexes in order to drive photosynthetic electron

transport. PSII antenna consists of at least six different chl-binding proteins (Ganeteg et al. 2004). LHCII, the major antenna complex of PSII is formed of trimers (Jansson 1994) of three proteins; Lhcb1, Lhcb2 and Lhcb3 in different combinations. The N-terminal threonine residue of the Lhcb1 and Lhcb2 proteins on the stromal side of the thylakoid membrane is prone to reversible phosphorylation (Bennett 1977) regulated by the redox conditions in the thylakoid membrane and the surrounding stroma (for a review see Aro and Ohad 2003). Three minor chl-binding proteins, Lhcb4 (CP29), Lhcb5 (CP26) and Lhcb6 (CP24), are monomeric, and among these proteins Lhcb4 is the only phosphoprotein in higher plants (Bergantino et al. 1995).

The PSII supercomplex is composed of a dimeric core complex of PSII, which binds two copies per dimer of each minor light-harvesting protein, two strongly bound LHCII trimers and one or more less tightly bound trimers (Boekema et al. 2000; Yakushevska et al. 2001; Dekker and Boekema 2005). The total amount of LHCII antennas is highly variable and strongly dependent on light conditions, the high light grown plants having less of antenna complexes (Anderson 1986; Bailey et al. 2001; Mäenpää and Andersson 1989). The Lhcb1 protein accounts for about 70% of LHCII (Jansson 1994, 1999), and it has been shown to respond most dynamically to the intensity of growth light (Bailey et al. 2001). Lhcb1 and Lhcb2 proteins, the mobile and phosphorylatable LHCII protein (Andersson et al. 2003), share high similarity in the sequence level and also in their physiological properties. Yet, there are some distinct differences—Lhcb1 has a higher chl *a*/*b* ratio (1.49 compared to 1.36 of Lhcb2) whereas Lhcb2 has somewhat lower carotenoid/chl ratio (Caffarri et al. 2004). A clear distinction in the biological roles of these two paralogous proteins remains unclear.

It is well known that LHCII is involved in short-term regulation of light harvesting, which occurs by two distinct mechanisms; the state transitions (Allen et al. 1981; Allen and Forsberg 2001) and the xantophyll and PsbS-dependent non-photochemical energy dissipation (NPQ) (Kulheim et al. 2002; for reviews see Demmig-Adams 2003; Niyogi et al. 2005). Although LHCII mainly functions as an antenna for PSII, it also harvests light for PSI due to state transitions induced by light intensity dependent Lhcb1 and Lhcb2 protein phosphorylation. The STN7 LHCII kinase is needed for catalysis of Lhcb1 and Lhcb2 protein phosphorylation (Bellafiore et al. 2005), the activation of the kinase being regulated by the redox state of the photosynthetic electron transfer chain (for reviews see Kruse 2001; Aro and Ohad 2003). Phosphorylation is activated by binding of reduced plastoquinone molecule to the Qo site of the cytochrome b_6f complex (Vener et al. 1997). Release of plastoquinone from the Qo site, in turn, deactivates the LHCII kinase. On the top of this regulation mechanism, an increase in stromal reductants exerts an inhibitory effect on the LHCII kinase, which occurs gradually, for example, upon increasing irradiance (Rintamäki et al. 2000; Hou et al. 2003). Accordingly, the LHCII kinase is deactivated or inhibited upon transfer of plants to darkness or suddenly to high light, respectively, both inducing state 1, whereas the kinase is active and state 2 generally dominates under low and/or moderate light regimes (Rintamäki et al. 2000). It is believed that such a reversible movement of LHCII balances the excitation pressures of PSII and PSI upon sudden changes in light conditions (Bennett et al. 1980; Horton and Black 1981).

State transition is a short-term acclimation process, which has been considered to happen in the timescale of minutes. Long-term light acclimation, on the contrary, takes days or even weeks and involves changes in the amounts of antenna proteins associated with PSII and PSI, as well as in the stoichiometry of the reaction centre complexes (for review see Anderson et al. 1995; Bailey et al. 2001). Here we studied the role of state transitions and the STN7 kinase in long-term acclimation of *Arabidopsis* to low (LL), moderate (ML) and relatively high light (HL) conditions. It is shown that the lack of the STN7 kinase and state transitions greatly modify the thylakoid protein contents upon acclimation of plants to LL and ML illumination conditions, however, with no obvious effect on the growth rate of plants. When the LL/ML growth conditions were interrupted by a short term illumination peak, the *stn7* mutant reacted more sensitively in enhancing the expression of stress-responsive genes, which probably causes retarded growth of the *stn7* mutant plants under fluctuating growth light conditions (Bellafiore et al. 2005).

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana (L.) ecotype Columbia (Col-0) was used for all experiments. Plants were grown in phytotron under different light intensities (30, 100 or 500 $\mu mol\ m^{-2}\ s^{-1}$), 8 h photoperiod, 23°C and relative humidity 70%. OSRAM PowerStar HQIT 400/D Metal Halide Lamps served as a light source.

Mature rosette leaves from 5 to 12 weeks old plants (HL plants 5–6 weeks, ML plants 7–8 weeks and LL plants 10–12 weeks) were used for experiments. The T-DNA insertion line in the Columbia background for the *STN7* gene (At1g68830) (SALK 073254) (Bellafiore 2005; Bonardi 2005) was purchased from the Salk Institute (Alonso et al. 2003). PCR analysis was performed to confirm that the mutant was homozygous. Primers were designed for the left (LP: aagccaattggtgaatcgctg) and right (RP: caacacttgctggtttgatgcag) flanking sequences of the T-DNA insert inside the *STN7* gene and for the T-DNA insert (LB: gcgtggaccgcttgctgcaact).

Short-term light treatments of plants

PSI and PSII light treatments. Plants were exposed for 3 h to light favouring the excitation of PSII (PSII light) and that of PSI (PSI light) to ensure the maximal phosphorylation and dephosphorylation of thylakoid proteins, respectively. A fluorescent tube (GroLux F58W/GROT8 Sylvania) covered with orange filter (Lee 105 filter, Lee Filters) served as PSII light and PSI light was obtained from halogen lamps (500 W) covered with an orange filter (Lee 105, Lee Filters) and a 'Median blue' filter (Roscolux # 83, Rosco Europe). Temperature was maintained at 23°C by water-cooled glass chamber between the fluorescence tube and the plants.

Short-term high light treatments. Intact plants (or detached leaves when indicated) were subjected to illumination by OSRAM PowerStar HQIT 400/D Metal Halide Lamps. The plants (or leaves) were placed in a temperature-controlled chamber at 23°C and light was passed through heat filter. Light intensities were adjusted by changing the distance between the lamp and the samples.

Lincomycin treatment of leaves. Lincomycin (1 mM) treatment was given through cut petioles over night in darkness, followed by floating the leaves in the same solution for 2 h under GL (30, 100 or 500 μmol photons m^{-2} s^{-1}) or under the short term high light conditions.

Isolation of thylakoid membranes, SDS-PAGE, protein staining and immunoblotting

Thylakoid membranes were isolated as described in Suorsa et al. (2004). Isolated thylakoids were solubilised in the presence of 6 M urea, and the polypeptides were separated by SDS-PAGE using 15% (w/v) acrylamide gels with 6 M urea (Laemmli 1970). ProQ Diamond Phosphoprotein gel stain and SYPRO® Ruby Protein Gel Stain (Molecular Probes) were used according to manufacture's instructions after determining the sample concentrations that give a linear response after staining. Thylakoids equivalent to 0.5 μg chl were loaded in gels to investigate the most abundant proteins and 3 μg chl was loaded to investigate the less abundant thylakoid proteins.

For immunoblotting the amount of chl loaded in gels was tested for each antibody to give a linear response and varied between 0.3 and 6.0 μg chl depending on the antibody. The polypeptides were transferred to an Immobilon-P membrane (Millipore, Bedford, MA), and the membrane was blocked with 5% (w/v) milk (Bio Rad) or 5 % fatty-acid free bovine serum albumin (Sigma-Aldrich) for p-thr antibody (New England Biolabs). Western blotting was performed with standard techniques using protein-specific antibodies purchased from Agrisera (Lhcb1, Lhcb2, Lhcb3, CP26 (Lhcb5), CP24 (Lhcb6), PsaG, Lhca1, Lhca2, Lhca3, Lhca4, PsbS) and other antibodies as described previously (Allahverdiyeva et al. 2005; Suorsa et al. 2004). Proteins were immunodetected using a Phototope-Star Chemiluminescent kit (New England Biolabs).

Mass spectrometry analysis of phosphopeptides

The surface-exposed peptides were cleaved from the thylakoids with trypsin. Isolated thylakoids were first resuspended in 25 mM NH_4HCO_3, 10 mM NaF to a final concentration of 3 mg of chl/ml and incubated with mass spectrometry grade-modified trypsin (Promega) (5 μg enzyme/mg chl) for 3 h at 22°C. The digestion products were frozen, thawed and centrifuged at 15,000 *g*. The supernatant was collected while the membranes were resuspended in water and centrifuged again. Both supernatants containing released thylakoid peptides were pooled and centrifuged at 100,000 *g*.

For isolation of phosphopeptides, the peptides released by trypsin were lyophilized and methyl-esterified with 2 N methanolic HCl (Ficarro et al. 2002). Phosphopeptides were enriched by IMAC as described (Vainonen et al. 2005). Phosphopeptides were eluted by 4 × 10 μl of 20 mM Na_2HPO_4 with 20% acetonitrile and desalted using POROS R3 (PerSeptive Biosystems). The mass spectra were acquired on a hybrid mass spectrometer API QSTAR Pulsar i (Applied Biosystems) equipped with a nanoelectrospray ion source (MDS Protana). The nanoelectrospray capillaries were loaded with 2 μl of peptide solution in 50% acetonitrile, 1% formic acid in water. Mass spectra

were acquired with instrument settings recommended by Applied Biosystems.

77 K fluorescence emission spectra

Leaves were harvested for 77 K fluorescence emission measurements from different growth light conditions 3 h after the lights were turned on, or after 3 h illumination of plants under the PSII and PSI specific lights. After illumination, the leaves were immediately frozen in liquid nitrogen. Leaves were then grinded in buffer containing 50 mM Hepes/KOH, pH 7.5, 100 mM sorbitol, 10 mM $MgCl_2$, and 10 mM NaF. After filtration, the samples were diluted to a chl concentration of 10 μg/ml and the 77 K fluorescence emission spectra were immediately recorded with a diode array spectrophotometer (S2000; Ocean Optics, Dunedin, FL, USA) equipped with a reflectance probe. Fluorescence was excited with white light below 500 nm, defined by using LS500S and LS700S filters (Corion Corp., Holliston, MA, USA). The emission between 600 and 780 nm was recorded. More than ten independent measurements were made from each of differentially light treated WT and *stn7* plants.

Fluorescence measurements at room temperature

PSII efficiency was determined as a ratio of variable fluorescence(F_v) to maximal fluorescence (F_m) measured from intact leaves with a Hansatech Plant Efficiency Analyser (King's Lynn) after a dark incubation of 30 min.

Non-photochemical quenching of chl fluorescence (NPQ) was determined in intact leaves exposed to actinic light of 300 and 1500 μmol photons m^{-2} s^{-1}, provided by Halogen lamp using PAM-2000 Fluorometer and the DATA Acquisition Software DA-2000. Plants were placed in the dark for at least 1 h prior to measurement. The NPQ-parameter was calculated according to the equation: $NPQ = (F_m - F_m')/F_m'$.

PSII excitation pressure (1-qP) was measured with a PAM florometer (PAM 101/103, Heinz Walz) as described previously (Piippo et al. 2006).

DNA microarray experiments

Arabidopsis cDNA microarray chips are based on the GEM1 clone set purchased from IncyteGenomics, Palo Alto, CA, USA and contains circa 6500 unique genes (for details see Piippo et al. 2006). Each clone was spotted three times to an array to provide technical replicates.

The leaves acclimated to different growth irradiances and analysed with microarrays were collected 2 h after the onset of the lights. Similarly, the plants for short-term treatments were shifted from growth light to high light 2 h after the onset of lights. The microarray data for long-term acclimated plants is derived from three independent biological replicates, and data for high light treatments are derived from two independent biological replicates for all samples. Leaves of five plants were pooled to each biological sample. Total RNA was extracted with Trizol-reagent (Invitrogen, Carlsbad, CA, USA) and treated with RNase free DNase set (Qiagen, Hilden, Germany). 30 μg RNA was indirectly labeled with aminoallyl method as follows. Reverse transcription of RNA was performed with anchored d(T)20 primers (Invitrogen, Carlsbad, CA, USA) in the presence of aminoallyl-dUTP (Sigma, st. Louis, MA, USA) using SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) after which the fluorescent Cy3 or Cy5 NHS ester (Amersham Biosciences, Piscataway, NJ, USA) was coupled to samples. The microarray slides were UV-crosslinked (90 mJ/cm^2) and prehybridised with 1% BSA (Sigma, st. Louis, MA, USA). The labeled samples were combined and hybridized in a total volume of 80 μl at 65°C overnight under a LifterSlip (Erie Scientific Company, Porthsmouth, NH, USA). The arrays were scanned with Agilent's microarray scanner (Agilent Technologies, Palo Alto, CA, USA) and the spot intensities were quantified with Scan Array Express Microarray Analysis System 2.0 (Perkin–Elmer Life Sciences, Wellesley, MA, USA) using adaptive circle method. The data was normalized and analysed with GeneSpring 7.2 (Silicon Genetics, Redwood City, CA, USA).

Results

Thylakoid protein contents in *stn7* and WT plants with respect to growth irradiance

No visible phenotype was observed for the *stn7* plants grown under LL, ML and HL conditions.

It was therefore tested whether the lack of the STN7 kinase changes the thylakoid protein contents, in order to compensate the lack of state transitions (Depege et al. 2003; Bellafiore et al. 2005). To this end, an immunoblotting analysis was performed on the contents of representative proteins in different thylakoid protein complexes, the PSI and PSII complexes, ATP synthase, the $Cytb_6f$ and NDH-1 complexes, and the LHC antenna proteins of PSII and PSI as well as the

PsbS protein known to be essential in the development of NPQ (Fig. 1).

In WT plants the most obvious scheme in long-term acclimation to increasing irradiance was a decrease in the amounts of the Lhcb1 and Lhcb3 proteins and conversely, an increase in the PsbS protein. Only minor decrease was recorded for the Lhcb2 protein, and of the LHCI antenna proteins only Lhca4 showed significant down regulation with increasing growth light intensity (Fig. 1). These changes in antenna proteins were reflected in an increase in chl *a*/*b* ratio with increasing growth light intensity (Fig. 1C). It is notable that the *stn7* mutant had lower chl *a*/*b* ratio compared to WT under all growth conditions (Fig. 1C). The *stn7* kinase mutant also showed a distinct difference from WT in the thylakoid protein acclimation response, being incapable of adjusting the content of the Lhcb1 protein according to varying growth irradiance. In mutant plants, the Lhcb1 protein content remained at the level of only about 30–60% of that in WT depending on the growth light condition, the biggest difference to WT being manifested in LL grown *stn7* plants. On the contrary, the Lhcb2 protein, particularly the upper isoform of the two protein bands recognised by anti-Lhcb2, was present in higher amounts in the *stn7* mutant under LL and ML conditions as compared to WT thylakoids (Fig. 1A). Moreover, the α and β subunits of the chloroplast ATP synthase, the NdhI subunit of the chloroplast NDH-1 complex and the PSI antenna proteins Lhca1 and Lhca2 were strongly up-regulated under LL conditions in the mutant as compared to WT thylakoids. Also the PsbS content of the *stn7* mutant was higher than that in WT, particularly under ML and HL growth conditions.

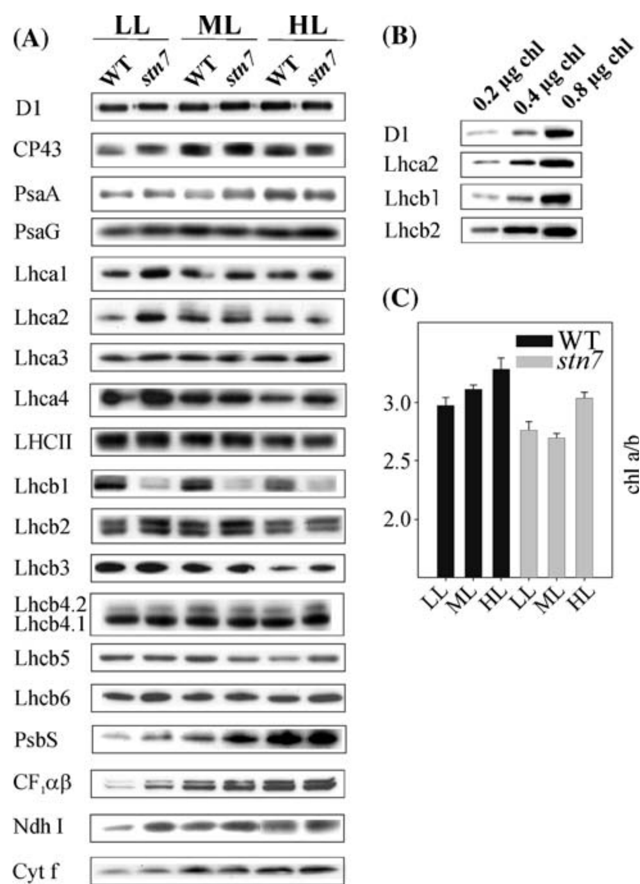

Fig. 1 Analysis of thylakoid membrane proteins and chl *a*/*b* ratios of the wild type (WT) and *stn7* mutant plants. (**A**) Immunoblots of thylakoid membrane proteins from WT and *stn7* mutant plants grown under LL (30 µmol photons m^{-2} s^{-1}), ML (100 µmol photons m^{-2} s^{-1}) and HL (500 µmol photons m^{-2} s^{-1}). Leaves were harvested for thylakoid isolation 3 h after the lights were turned on. 0.3–6.0 µg of chl were loaded in the wells according to the linearity tests with antibodies. (**B**) The linearity of immunoresponse with D1, Lhca2, Lhcb1 and Lhcb2 antibodies, as examples. (**C**) Chl *a*/*b* ratio in LL, ML and HL grown WT and *stn7* plants. Error bar represents the standard deviation of the mean

Due to different acclimation responses of the Lhcb proteins in the WT and *stn7* plants, as revealed by immunoblotting, the thylakoid membrane proteins were also stained with SYPRO® Ruby Protein Gel Stain after SDS-PAGE. These gels did not reveal a net loss of Lhcb proteins in the *stn7* mutant under any growth condition (Fig. 2A), indicating that the loss in Lhcb1 protein in the mutant was compensated with an increase in the amount of the Lhcb2 protein.

Thylakoid protein phosphorylation in differentially light acclimated WT and *stn7* plants

Although it has been previously demonstrated that the *stn7* kinase mutant is deficient in LHCII protein phosphorylation (Depege et al. 2003; Bellafiore et al. 2005; Bonardi et al. 2005), we performed the phosphoprotein analysis with both the p-thr antibody and the Pro-Q® Diamond Phosphoprotein Gel Stain to see whether the phosphorylation of other thylakoid phosphoproteins was affected as well. Moreover, the Pro-Q® Diamond Phosphoprotein Gel Stain reacts with the phospho-groups of proteins, thus giving more quantitative information of the relative phosphorylation of different phosphoproteins in a given sample, the fact that is impossible to evaluate from immunoblotting with p-thr antibodies (Aro et al. 2004; Schulenberg et al. 2003)

As expected, immunoblotting with p-thr antibody and Pro-Q® Diamond Phosphoprotein Gel Stain revealed no Lhcb1 or Lhcb2 protein phosphorylation in mutant thylakoids (Figs. 2, 3A). Importantly, the *stn7* mutant also lacked Lhcb4.2 (CP29) phosphorylation

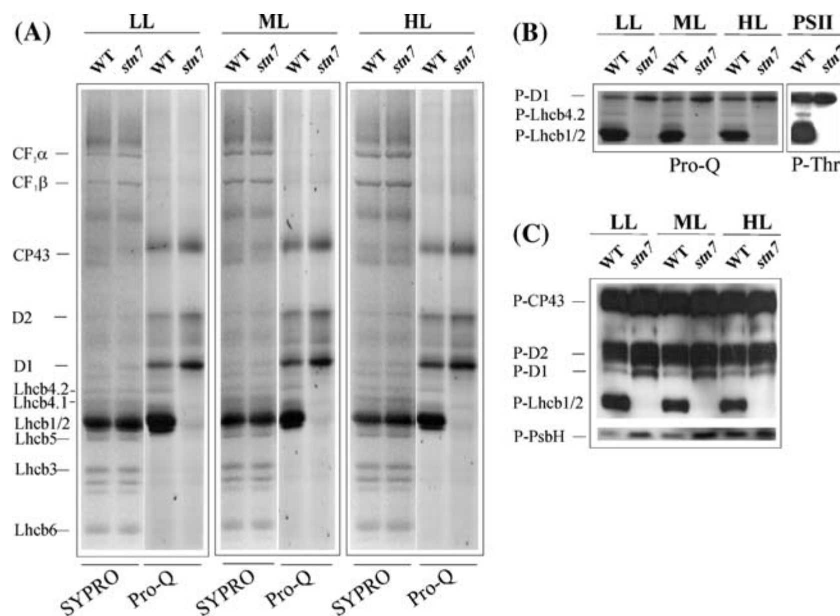

Fig. 2 Phosphorylation of thylakoid proteins in the WT and *stn7* mutant leaves. (**A**) SYPRO® Ruby Protein Gel Stain and Pro-Q® Diamond Phosphoprotein Gel Stain stained SDS-PAGE gels from the WT and *stn7* plants grown in LL (30 μmol photons m^{-2} s^{-1}), ML (100 μmol photons m^{-2} s^{-1}) and HL (500 μmol photons m^{-2} s^{-1}). Leaves for measurements were harvested 3 h after the lights were turned on. Protein identification is based on immunoblotting and/or mass spectrometry (data not shown). About 16 cm long gels were run until the 16 kDa protein standard was at the bottom of the gel. (**B**) Comparison of Lhcb phosphoproteins stained with Pro-Q® as in A and after 3 h illumination with PSII light and immunoblotting with p-thr antibody. *Stn7* mutant shows no phosphorylation of Lhcb4.2. Gels were run as in **A**. (**C**) P-thr blot of thylakoid proteins separated in mini-gels from plants grown under LL, ML and HL. About 3 μg of chlorophyll was loaded in **A** and **B** and 0.75 μg in **C**

under all different growth lights as well as after a 3 h PSII light treatment, which induced maximal STN7 kinase activation (Fig. 2B) and has previously been shown to induce also the phosphorylation of CP29 in rye leaves (Pursiheimo et al. 2003). To confirm that CP29 indeed is a target of the STN7 kinase, the presence of thylakoid phosphoproteins was confirmed by mass spectrometry. The mapping of phosphopeptides from *stn7* thylakoids in comparison with the known phosphopeptides from WT thylakoids (Vener et al. 2001; Hansson and Vener 2003) revealed the specific absence of phosphopeptide FGFGtKK with *m*/*z* 439.7^{2+} from the thylakoids of the *stn7* mutant. This phosphopeptide corresponds to Lhcb4.2 (At3g08940) where the Thr-6 is phosphorylated. The phosphopeptide Ac-RRtVK with *m*/*z* 398.2^{2+} was also absent in the *stn7* thylakoids. This short peptide originated from the products of At2g05100 and At2g05070 *Lhcb2* genes in *Arabidopsis*. All identified phosphopeptides isolated from the *stn7* and wild type thylakoids are presented in Table 1.

In WT plants, the strongest phosphorylation of LHCII proteins was recorded under steady state LL growth conditions. However, even under high growth irradiance the LHCII proteins were quite strongly phosphorylated due to the fact that under steady state growth conditions the stromal homeostasis is maintained, in contrast to sudden high light peaks that inducing strong reduction of the stroma, and inhibition of the STN7 kinase (Fig. 2A, C). Lhcb4.2 (CP29) was phosphorylated under similar conditions as the Lhcb1 and 2 proteins (Fig. 2B). By comparing the phosphoprotein (Pro-Q®) intensities it is clear that the Lhcb1/Lhcb2 proteins are the most abundant phosphoproteins in the thylakoid membrane. The phosphorylation intensity of the Lhcb4.2 protein is minimal under all different illumination conditions and even less than that of the PSII core proteins. On the other hand, the PSII core proteins CP43, D2, D1 and PsbH were all more strongly phosphorylated in the *stn7* mutant than in the WT plants independently of growth conditions. This is in accordance with higher PSII excitation pressure recorded in each growth condition for the *stn7* mutant leaves as compared to WT leaves (Table 2 and Bellafiore et al. 2005).

Comparison of thylakoid Lhcb protein phosphorylation and the 77 K fluorescence emission

WT and *stn7* mutant plants were illuminated for 3 h with PSI light, which dephosphorylates the thylakoid

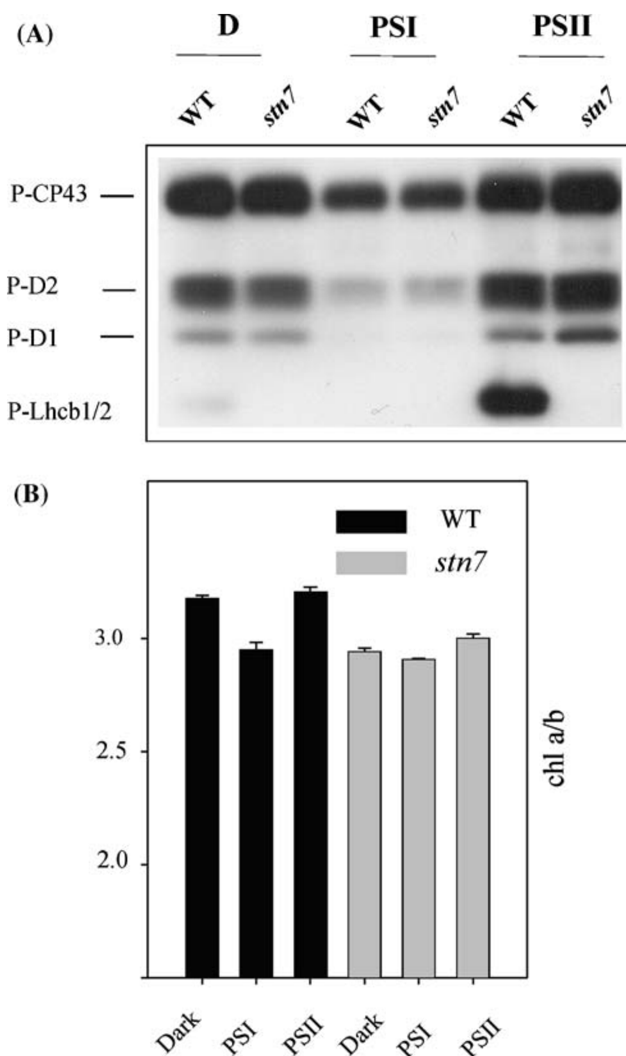

Fig. 3 Phosphorylation of thylakoid proteins and the chl *a*/*b* ratios of WT and *stn7* mutant leaves after short-term PSI and PSII light treatments. Dark samples were collected after 16 h in darkness (D) and the PSII light (60 μmol photons m^{-2} s^{-1}) and PSI light (50 μmol photons m^{-2} s^{-1}) treated samples were illuminated under respective light for 3 h after the diurnal dark period. (**A**) Phosphorylation of thylakoid proteins was determined by immunoblotting with p-thr antibody, 1.0 μg of chlorophyll was loaded in each well. (**B**) Chl *a*/*b* ratios. Error bar represents the standard deviation of the mean

phosphoproteins including Lhcb1 and Lhcb2 and conversely, with PSII light to fully phosphorylate the respective proteins (Fig. 3; Hou et al. 2003). Plants for these experiments were all grown under ML. PSI and PSII light treated plants were then subjected to 77 K fluorescence emission measurements to evaluate the maximal extent of state transition induced by Lhcb protein phosphorylation (Fig. 4). The 77 K fluorescence emission ratio F733/F685 was calculated as an indication of energy distribution between PSI and PSII. Upon illumination of WT plants with PSI light the ratio was as low as 0.9 (Fig. 4A), describing the maximal state 1 in these plants. In PSII light treated WT plants the high phosphorylation level of LHCII proteins (Fig. 3) clearly induced a transition to state 2, which was reflected in high F733/F685 ratio of 1.7 ± 0.2. The *stn7* kinase mutant, on the contrary, showed a lack of both the LHCII protein phosphorylation (Fig. 3) and the state transitions (Fig. 4B), and the 77 K fluorescence emission ratio F733/F685 was maintained at around 1.0 irrespectively whether the plants were preilluminated with PSI or PSII light.

It is intriguing to note that the 3-h treatment of plants under PSI light also lowered the chl *a*/*b* ratio in WT thylakoids, whereas no such adjustments in the chl *a*/*b* ratio of *stn7* thylakoids were recorded (Fig. 3B).

Next, the 77 K fluorescence spectra were recorded from WT and *stn7* plants grown under LL, ML and HL conditions. WT plants (Fig. 4C) clearly maintained state 2 under LL growth condition (F733/F685 = 1.7 ± 0.2), and only slightly lower fluorescence emission ratio (F733/F685 = 1.5 ± 0.2) was recorded for ML growth conditions. HL growth conditions reduced the steady state F733/F685 fluorescence emission ratio to below 1.5, indicating an intermediate state between state 1 and state 2 (illumination of these HL plants with PSII light induced the F733/F685 ratio of 1.9 indicating that the capacity for state transitions was preserved even at HL).

77 K fluorescence emission measurements from *stn7* mutant thylakoids showed less flexibility, the F733/F685 ratios being 1.3, 1.0 and 1.1 in thylakoids from LL, ML and HL growth conditions, respectively (Fig. 4D). All these ratios were lower than the corresponding WT values. Thus, the *stn7* mutant showed distinct difference from WT plants in long-term light acclimation. The overall acclimation response of *stn7* plants, evident at all growth light conditions, resembled the HL acclimation strategy in WT *Arabidopsis*, i.e. a more or less equal distribution of excitation energy between the two photosystems, as interpreted from the 77 K fluorescence emission spectra.

Susceptibility of the *stn7* mutant to photoinhibition of PSII

The photochemical efficiency of PSII (F_v/F_m) was recorded from WT and *stn7* mutant plants exposed to different intensities of photoinhibitory irradiation for 2 h (Fig. 5). No differences in the susceptibility to photoinhibition were observed between LL grown WT and mutant plants. The PSII centres of ML and HL grown *stn7* plants were, on the contrary, more tolerant against high light stress than WT. When protein

Table 1 Phosphopeptides isolated from *stn7* and WT thylakoids and identified by ESI-MS

WT thylakoids			*stn7* mutant thylakoids		
Ion	Peptide	Protein	Ion	Peptide	Protein
738.4 $^{1+}$	Ac-tIALGK	D2	738.4 $^{1+}$	Ac-tIALGK	D2
852.4 $^{1+}$	Ac-tAILER	D1	852.4 $^{1+}$	Ac-tAILER	D1
504.7 $^{2+}$	Ac-tAILERR	D1	504.7 $^{2+}$	Ac-tAILERR	D1
439.7 $^{2+}$	FGFGtKK	CP29			
608.3 $^{2+}$	AtQTVEDSSR	psbH	608.3 $^{2+}$	AtQTVEDSSR	psbH
648.3 $^{2+}$	AtQtVEDSSR	psbH	648.3 $^{3+}$	AtQtVEDSSR	psbH
685.8 $^{2+}$	Ac-tLFDGTLALAGR (-1 CH_3)	CP43	685.8 $^{2+}$	Ac-tLFDGTLALAGR (-1 CH_3)	CP43
692.8 $^{2+}$	Ac-tLFDGTLALAGR	CP43	692.8 $^{2+}$	Ac-tLFDGTLALAGR	CP43
685.3 $^{2+}$	Ac-tLFNGTLALAGR	CP43	685.3 $^{2+}$	Ac-tLFNGTLALAGR	CP43
398.2 $^{2+}$	Ac-RRtVK	LHCII			
922.8 $^{3+}$	ATtEVGEAPATTTEAETTELPEIVK	TMP14	922.8 $^{3+}$	ATtEVGEAPATTTEAETTELPEIVK	TMP14

Table 2 PSII excitation pressure of WT and *stn7* plants under different growth light conditions

Growth condition	1-qP	
	WT	Stn 7
LL	0.13 ± 0.03	0.24 ± 0.02
ML	0.14 ± 0.02	0.18 ± 0.04
HL	0.27 ± 0.03	0.32 ± 0.02

The values are means ± SD. Measurements were performed under respective growth light conditions. LL 30 μmol photons m^{-2} s^{-1}, ML 100 μmol photons m^{-2} s^{-1} and HL 500 μmol photons m^{-2} s^{-1}

synthesis, and thus the repair of PSII centres was inhibited with lincomycin during the photoinhibitory illumination (Fig. 5), the PSIIs of the *stn7* mutant turned out to be even more tolerant against photodamage, as compared to WT. It is therefore conceivable that the light-harvesting and/or the quenching (NPQ) properties of the LHCII antenna systems differ between the *stn7* mutant and WT, possibly being related to different PsbS, Lhcb1 and Lhcb2 protein contents of the LHCII antenna and their phosphorylation (Fig. 1).

To get further insights in what happens in the *stn7* and WT plants when exposed to high light, we took plants grown under ML conditions, exposed them after the diurnal dark period to 500 μmol photons m^{-2} s^{-1} (light intensity that saturates CO_2 fixation in ML grown plants, data not shown) and collected leaf samples in the course of illumination to measure the F_v/F_m ratio, the 77 K fluorescence emission spectra and the Lhcb1/2 protein phosphorylation. As shown in Fig. 6, the decrease in F_v/F_m (measured after 30 min dark incubation) in WT plants in the course of illumination was accompanied by oscillations of the F733/F685 fluorescence emission ratio, which in turn was reflected in the phosphorylation of the Lhcb1/2 proteins. Quite drastic changes in the two latter parameters, indicating fluctuations from state 1 towards state 2 and back again, took place during the first 4 h of illumination, thylakoids then gradually balancing to an intermediate state. If, however, the WT plants were

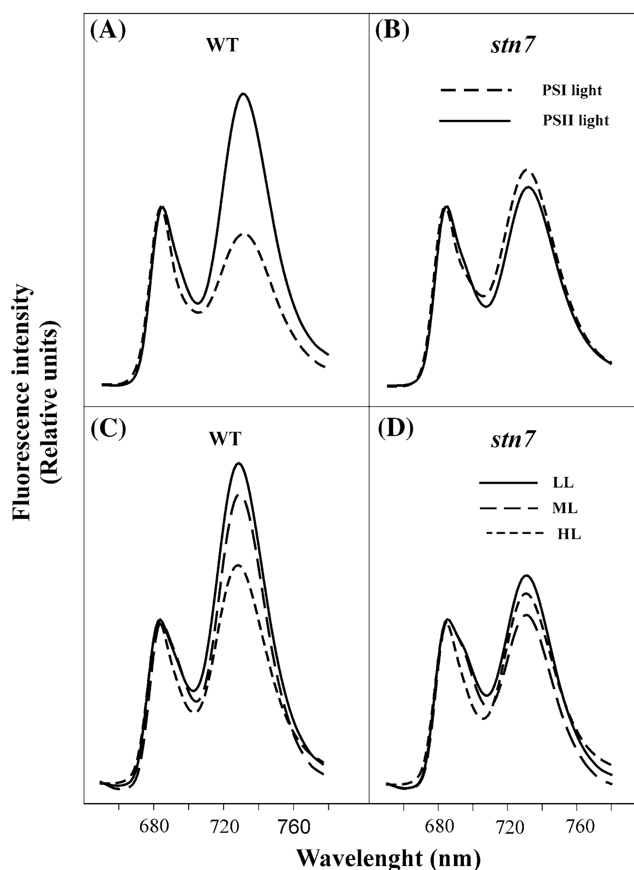

Fig. 4 77 K fluorescence emission spectra of WT and *stn7* mutant. (**A**) and (**B**). Fluorescence spectra were recorded after in vivo illumination of plants with PSI and PSII lights as described in Fig. 3. (**C**) and (**D**) the 77 K fluorescence spectra from plants grown in LL (30 μmol photons m^{-2} s^{-1}), ML (100 μmol photons m^{-2} s^{-1}) and HL (500 μmol photons m^{-2} s^{-1}). Leaves were harvested for measurements after 3 h of turning the lights on. Thylakoids were isolated in the presence of 10 mM NaF. 77 K fluorescence spectra were normalized at 685 nm fluorescence

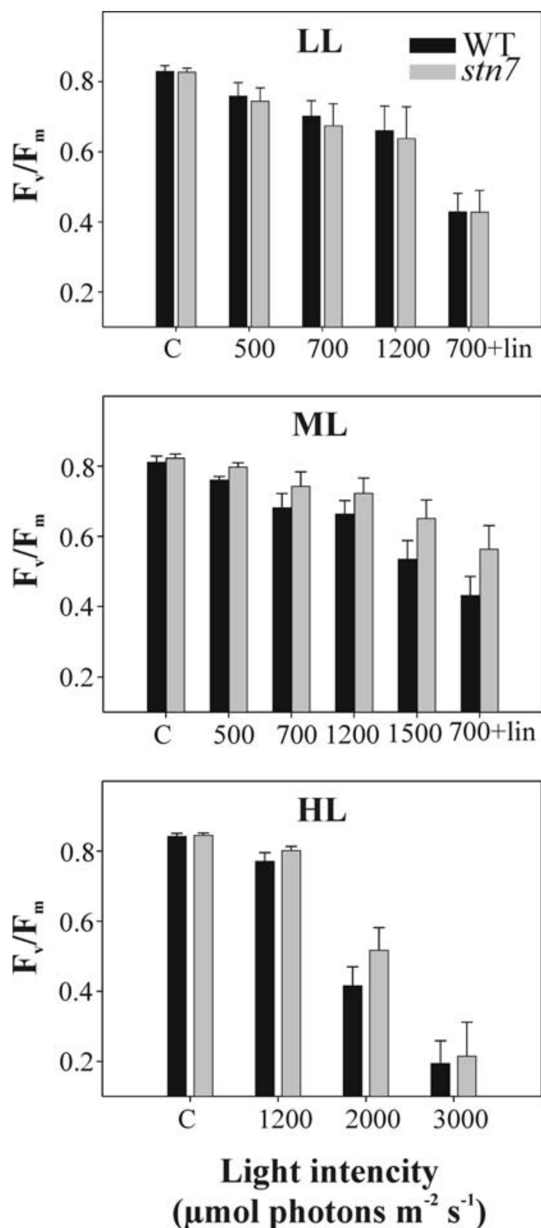

Fig. 5 Susceptibility of the WT and *stn7* mutant plants to photoinhibition of PSII. Leaves detached from plants grown in LL (30 µmol photons m^{-2} s^{-1}), ML (100 µmol photons m^{-2} s^{-1}) and HL (500 µmol photons m^{-2} s^{-1}) were exposed to high light treatments of different intensities as denoted below the bars, in the presence (lin+) and absence of lincomycin for 2 h. Control plants from GL are denoted as **C**. F_v/F_m was measured with Hansatech Plant Efficiency Analyser fluorometer after 30 min dark adaptation of leaves

exposed to 1000 µmol photons m^{-2} s^{-1} or higher light intensities, a direct shift to state 1 took place (data not shown). The *stn7* mutant grown under similar ML light conditions, on the contrary, showed only minor decrease in F_v/F_m and very small fluctuations in the F733/F685 ratio in the course of the 8 h illumination period under 500 µmol photons m^{-2} s^{-1}.

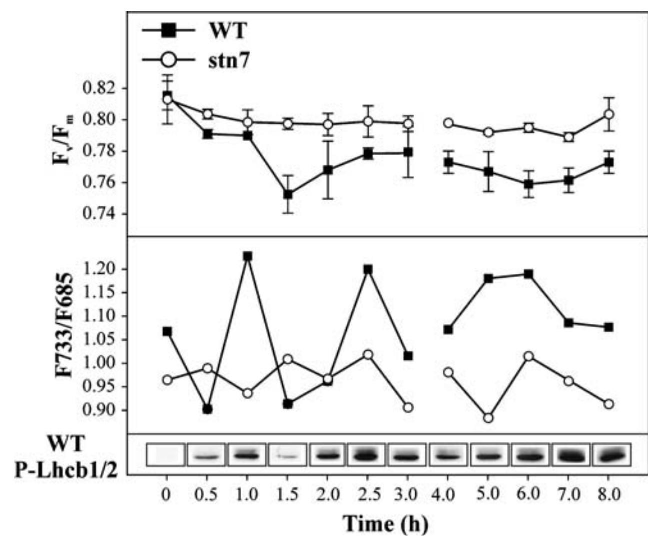

Fig. 6 Changes in the photochemical efficiency PSII (F_v/F_m), the 77 K fluorescence emission ratio F733/F685 and the LHCII protein phosphorylation in the course of 8 h illumination of WT and *stn7* mutant leaves under a photon flux density of 500 µmol photons m^{-2} s^{-1}. ML (100 µmol photons m^{-2} s^{-1}) grown dark-adapted (16 h) WT and *stn7* plants were exposed to illumination and samples were collected every 30 min and later every 60 min for measurements. F_v/F_m was measured with Hansatech Plant Efficiency Analyser fluorometer after 30 min dark adaptation of leaves. F733/F685 was calculated from 77 K fluorescence emission spectra. Phosphorylation of LHCII was determined from WT and *stn7* (no detectable phosphorylation) by immunoblotting with p-thr antibody, 0.75 µg of chl was loaded in each well. All the thylakoids were isolated in the presence of 10 mM NaF. Results shown are representative of two similar experiments

Capacity for NPQ in WT and *stn7* plants

To define whether the *stn7* mutation affects the thermal energy dissipation, the NPQ measurements were performed at two actinic light intensities (300 and 1500 µmol photons m^{-2} s^{-1}) (Fig. 7). Interestingly, the LL grown *stn7* mutant had clearly lower capacity for NPQ than LL grown WT plants, recorded both at 300 and 1500 µmol photons m^{-2} s^{-1}. On the contrary, the ML and HL grown *stn7* plants showed higher NPQ at both actinic light intensities as compared with WT. Moreover, the NPQ under these conditions persisted at higher level for the *stn7* plants also under prolonged light stress (data not shown).

Transcript analysis of the *stn7* kinase mutant and WT after long- and short-term light treatments

Differential expression observed for several thylakoid proteins between the *stn7* and WT plants (Fig. 1) prompted us to investigate whether the STN7 kinase exerts its effect on the transcriptional regulation of *Arabidopsis* nuclear genes in our experimental condi-

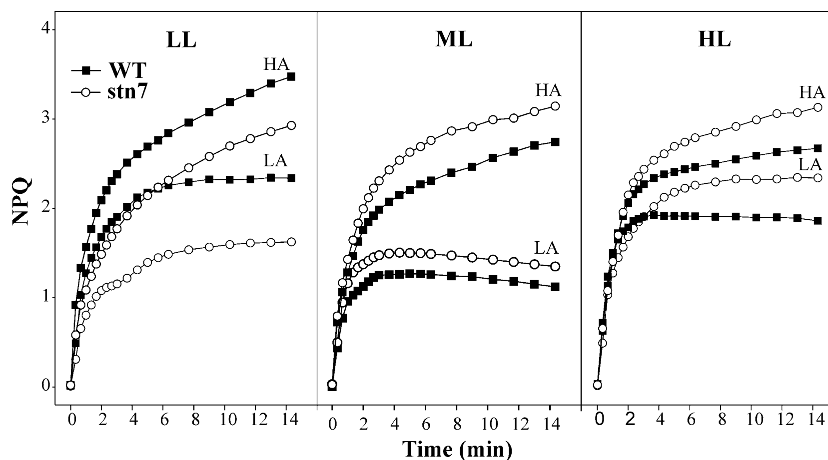

Fig. 7 Induction of NPQ in the WT and *stn7* mutants plants. NPQ was measured from plants grown in LL (30 µmol photons m^{-2} s^{-1}), ML (100 µmol photons m^{-2} s^{-1}) and HL (500 µmol photons m^{-2} s^{-1}) by using two different actinic light intensities 300 (LA) and 1500 µmol photons m^{-2} s^{-1} (HA)

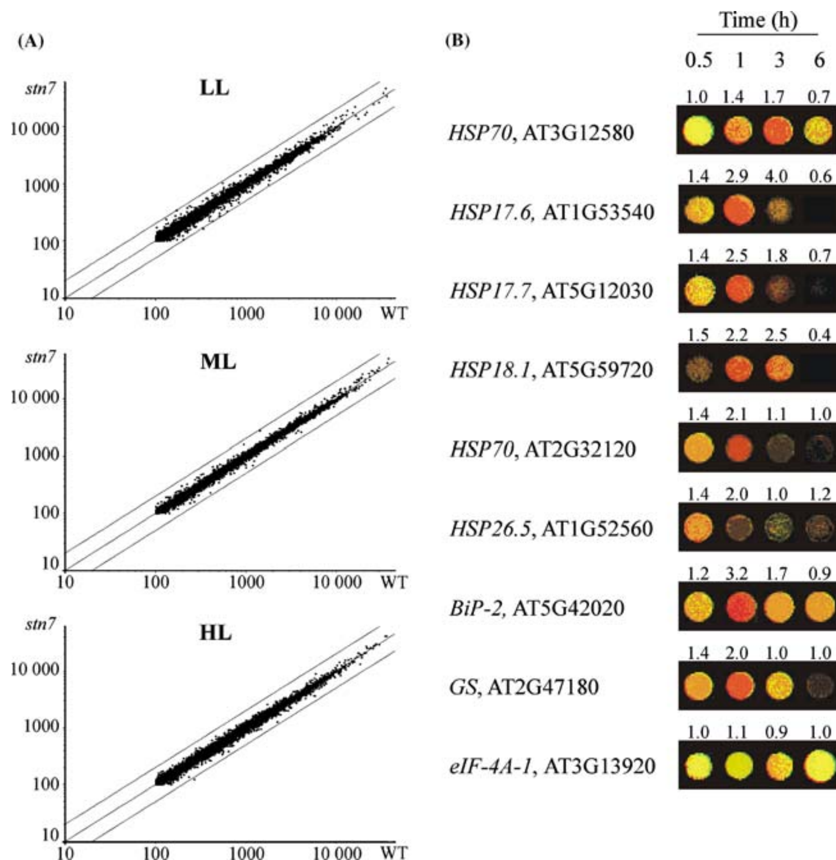

Fig. 8 Comparison of gene expression between WT and *stn7* leaves under steady state growth conditions and after short-term excess light treatments. (**A**) Scatter plots of signal intensities of LL (30 µmol photons m^{-2} s^{-1}), ML (100 µmol photons m^{-2} s^{-1}) and HL (500 µmol photons m^{-2} s^{-1}) grown WT plants versus *stn7* mutant plants subjected to 8 K microarray analysis. None of the differences in signal intensities between WT and *stn7* were statistically significant. (**B**) ML grown WT and *stn7* plants were exposed to 1200 µmol photons m^{-2} s^{-1} for 0.5, 1, 3, and 6 h. RNA was isolated and subjected to an 8 K cDNA microarray. Spots of genes revealing distinct differences in expression between WT and *stn7* are depicted in the figure. Eucaryotic translation initiation factor (eIF–4A–1) is shown as a control for spot colours and shows fairly balanced scanning of both fluorophores. Red denotes higher expression of the gene in *stn7* than in WT. Yellow indicates equal expression of the gene in WT and *stn7*. Expression fold changes, *stn7* versus WT, of the normalized data are given on the top of each spot. Results are representative of two independent experiments. GS = galactinol synthase, BiP-2 = luminal binding protein 2

tions. To get a holistic picture about modulations in the transcriptome by *stn7* mutation, we subjected the RNA isolated from *stn7* mutant and WT leaves to an 8 K cDNA array (Piippo et al. 2006). Interestingly, the arrays did not reveal any statistically significant gene expression differences between the *stn7* mutant and WT plants grown under LL, ML or HL conditions (Fig. 8A) suggesting a similar cellular homeostasis upon long-term light acclimation of *stn7* and WT *Arabidopsis* plants. These results indicate that the STN7 kinase activity as such has no direct effects on nuclear gene transcription in *Arabidopsis*.

Interestingly, when the plants grown under ML conditions were suddenly exposed to strong light of 1200 μmol photons m^{-2} s^{-1} for a short time period from 30 min to 6 h, clear differential gene expression was recorded between the *stn7* and WT leaves in several stress responsive genes, particularly in various heat shock protein genes (*HSP*), *BiP–2* and *GS* genes as depicted in Fig. 8B. These differentially expressed stress genes showed 2–4-fold higher expression in *stn7* upon exposure of leaves to high light as compared to WT plants. The upregulation of most stress genes both in *stn7* and WT plants was only transient and distinct downregulation occurred within 6 h of high light exposure, in most of the stress genes already within 3 h of high light exposure.

Discussion

Photosynthetic light acclimation is a complicated mixture of long- and short-term processes. Short-term acclimation includes a series of dynamic processes like reversible phosphorylation of thylakoid membrane proteins, state transitions, quenching of excitation energy as heat, and fine-tuning the distribution of electrons between linear and cyclic electron transfer routes (for a review see Kanervo et al. 2005). Long-term acclimation, in turn, involves changes in the expression of photosynthetic genes, resulting in an asymmetric synthesis of photosynthetic proteins and protein complexes (Anderson et al. 1995; for a review see Walters 2005). A recently characterized *stn7* kinase mutant of *Arabidopsis* (Bellafiore et al. 2005), incapable of LHCII phosphorylation and state transitions, provides an interesting tool to study whether any regulatory networks exist between the *stn7* kinase induced short-term processes and the long-term acclimation processes of chloroplasts.

Lhcb4 (CP29) is also a target of the STN7 kinase

An important observation was that the phosphorylation of the Lhcb4.2 protein, a minor LHCII protein that together with a non-phosphorylatable Lhcb4.1 protein constitutes *Arabidopsis* CP29, is also under the control of the STN7 kinase. All the phosphoprotein analysis methods, p-thr antibody, Pro-Q® -staining and mass spectrometry revealed Lhcb4.2 phosphorylation in WT thylakoids but not in the thylakoids of the *stn7* mutant. Apparently the phosphorylation site of Lhcb4 proteins and also the phosphorylation kinetics vary depending on plant species (Bergantino et al. 1998), making it difficult to assess the physiological significance of Lhcb4 protein phosphorylation. The phosphorylation pattern of Lhcb4.2 followed that of the Lhcb1 and Lhcb2 proteins under varying light conditions suggesting that Lhcb4.2 phosphorylation might be important for the mechanism of state transitions in *Arabidopsis*, as also recently suggested for *Chlamydomonas* (Kargul et al. 2005). From Pro-Q®-stainings it is clear that the phosphorylated Lhcb4.2 protein is present only in a small fraction of the PSII-LHCII supercomplexes, and is not ''hyper-phosphorylated'' as reported for *Chlamydomonas*. It is conceivable that the Lhcb4.2 protein phosphorylation occurs only in those PSII-LHCII supercomplexes that participate in state transitions, thereby modulating the forces in the PSII-LHCII supercomplexes in the grana and resulting in the migration of a portion of LHCII from the PSII core to the stroma thylakoids in order to serve the light harvesting function for PSI.

Lack of the STN7 kinase modulates the LL and ML response of several thylakoid proteins

Asymmetric antenna protein synthesis, a typical response in long-term acclimation (Anderson and Osmond 1987; Anderson et al. 1995; Walters 2005), together with the capacity for state transitions allowed drastic modulations in the distribution of excitation energy between the two photosystems in WT thylakoids upon changes in light conditions (Fig. 4A, C). Characteristic of *stn7*, on the contrary, was a very low content of the Lhcb1 protein even under LL conditions, this resembling the acclimation strategy of WT plants to HL conditions. Indeed, besides the lack of state transitions, the *stn7* plants differed from WT plants also in the adjustment of the light harvesting antenna systems during long-term acclimation to various light regimes. *stn7* mutant was characterized by a much higher Lhcb2/Lhcb1 ratio upon growth under LL as compared to WT. Interestingly, under LL conditions, the *stn7* mutant also had lower capacity for NPQ compared to WT. As the PsbS contents were low both in the *stn7* and WT plants under LL, it is conceivable that the Lhcb proteins have different properties with

respect to non-photochemical energy quenching, the Lhcb2 being less efficient than Lhcb1 (Bailey et al. 2004). Under steady state ML and HL growth conditions, the higher PsbS protein content in the *stn7* mutant was reflected in higher NPQ and a higher tolerance against photoinhibition of PSII (Fig. 5) as compared to WT. Although it is well known that PsbS is required for the qE type of NPQ (Li et al. 2000), our results with LL grown *stn7* and WT plants strongly support the recent suggestion that the Lhcb antenna proteins also have a PsbS-independent role in the formation of NPQ (Dall'Osto et al. 2005).

stn7 mutant has also a lowered capacity to adjust the chl *a*/*b* ratio (Bonardi et al. 2005) both upon short- and long-term light acclimations as compared to WT (Figs. 1, 3). As a short-term response to changing light quality, the WT plants showed a distinct drop in the chl *a*/*b* ratio during the 3-h exposure to PSI light, and this change was not related to changes in protein content (data not shown). Thus, a yet uncharacterised mechanism exists behind such a short-term chl *a*/*b* change (under investigation, unpublished data). The physiological role of this short-term mechanism could be the adjustment of the light absorption window of the two photosystems to respond to the requirements of either the state 1 or state 2. Notably, *stn7* has lost nearly all the capacity for this short-term chl *a*/*b* adjustment upon changing light quality conditions (Fig. 3). The long-term changes in chl *a*/*b* ratio strongly rely on differential synthesis of the light-harvesting pigment protein complexes, which in *stn7* was more limited than in the WT plants.

Differences in the long-term acclimation between *stn7* and WT thylakoids were not limited only to the proteins and pigments associated with the function of PSII light harvesting. Indeed both the Lhca1 and Lhca2 light harvesting proteins of PSI were up regulated under LL growth conditions in *stn7* as compared to WT. This is an apparent compensation mechanism to increase the relative light absorption by PSI, which in WT thylakoids occurs via phosphorylated Lhcb proteins upon transition to state 2 under LL and ML conditions. Moreover, a drastic up regulation specifically of the CF_1 α and β subunits of the ATP-synthase and of the NdhI subunit of the NDH-1 complex was detected under LL and ML growth conditions in *stn7* compared to WT (Fig. 1, similar results were obtained when gels were loaded on protein basis, data not shown). These are typical HL acclimation responses reported for WT plants (Chow and Anderson 1987; Endo et al. 1999), and the increase in the NDH-1 complex also suggests modifications in the distribution of electrons between the linear and cyclic electron transfer routes in *stn7* as compared to WT.

How does the lack of the STN7 kinase induce such drastic and specific modifications in the thylakoid protein contents upon steady state growth conditions? From our DNA microarray experiments it is clear that the STN7 kinase activity as such does not maintain any differential gene expression at the level of transcription (Fig. 8A). Indeed, under steady state growth conditions of different light intensities, the nuclear gene transcription in *stn7* was similar to that in WT *Arabidopsis* (Fig. 8A). This also suggests that the cellular homeostasis similar to that in WT is reached in the *stn7* mutant by modulation of the thylakoid protein composition (Fig. 1) and maintains steady and balanced signalling for nuclear gene expression, like in WT plants. In line with several recent reports concerning the regulation of LHCII proteins (Mussgnug et al. 2005; Zelisko et al. 2005), we conclude that the difference in the thylakoid protein contents between *stn7* and WT plants under the steady state growth conditions occurs preferentially at the post-transcriptional level.

State transitions—a feedback mechanism to control the excitation of PSI

We show that the *stn7* mutant, incapable for state transitions, modifies the thylakoid protein composition such that the excitation of the two photosystems occurs more or less evenly independently of the growth light intensity (Fig. 4D). When the *stn7* mutant was grown under LL, ML or HL condition, or shortly treated with either the PSI or PSII light, the 77 K fluorescence emission ratio, F733/F685, always remained around 1. On the contrary, the WT plants exhibited big differences in F733/F685 ratio, ranging from below 1 up to 1.9, when the LHCII proteins were fully dephosphorylated or fully phosphorylated, respectively. Upon steady state growth under low or high light intensities, WT *Arabidopsis* tends to keep the LHCII proteins nearly maximally phosphorylated during the light phase thus maintaining state 2. WT plants thus seem to rely under standard growth conditions on relatively stronger excitation of PSI than PSII and thereby on a low excitation pressure on PSII (Table 2). It is worth noting that under all three different growth conditions, the WT plants preserved nearly similar capacity to perform state transitions (data not shown; Bailey et al. 2004).

A shift of leaves to substantially higher light intensity than generally experienced during the growth, induced fluctuations in state transitions (Fig. 6), most

probably to keep PSI at optimal operation but yet avoiding the over reduction of stroma, which would initiate the production of reactive oxygen species (Foyer and Noctor 2005). It is noteworthy that such a buffering system against stromal over reduction by transition from state 2 to state 1 occurs by a feedback mechanism i. e. the stromal reductants inhibit the phosphorylation of LHCII (Hou et al. 2003). Under sudden high light peak, however, the stroma becomes rapidly fully reduced, inducing prompt inactivation of the LHCII kinase (Rintamäki et al. 2000) and locking of LHCII to PSII. Indeed, the WT plants use the LHCII protein phosphorylation to dynamically regulate the distribution of excitation energy between the two photosystems according to environmental cues (Allen et al. 1981; Allen and Forsberg 2001), and we postulate that this flexibility is particularly important during the transient high irradiance peaks, which induce an inhibition of the STN7 kinase.

We did not find any special phenotype for the *stn7* plants when grown under steady state conditions of different light intensities. Thus the plant growth rate was not compromised under steady illumination conditions, whether the distribution of excitation energy favoured PSI (LL and ML grown WT plants) or whether the excitation was more evenly distributed between the two photosystems in the absence of state transition (LL and ML grown *stn7* mutants). This must be a consequence of the marvellous capacity for re-modelling of the thylakoid protein composition and possibly also of the electron transfer routes in *stn7* as discussed above. Other type of state transition mutants have also been shown to have compensation mechanisms for this defect (Lunde et al. 2003). A compromise is likely to become evident only when the transition to state 1 would be beneficial upon a high light illumination peak under fluctuating light environment. WT plants can easily shift to state 1 by inhibition of LHCII phosphorylation upon accumulation of reducing equivalents in the stroma, the phenomenon that can be mimicked by feeding the leaves when in state 2 with thiol reductants or sugar compounds (Rintamäki et al. 2000; Hou et al. 2003). Such a shift naturally accompanies an increased excitation of PSII. However, any harmful effects on PSII can be avoided by efficient feed back de-excitation in PSII and its LHCII antenna (Horton et al. 1996; Demmig-Adams 2003; Niyogi et al. 2005) or by turnover of the D1 protein upon photoinhibition of PSII (Aro et al. 1993). It is highly conceivable that transition to state 1 functions as a buffering system to avoid the over-reduction of chloroplast stroma and possibly also the photoinhibition of PSI which, in contrary to PSII photoinhibition, is an irreversible phenomenon with no rapid repair mechanism (Scheller and Haldrup 2005). The *stn7* mutant lacks this flexibility and—independently of growth light intensity—acquires a partial ''high light'' acclimation strategy.

State transitions control the extent of stress protein expression

A retarded growth of the *stn7* mutant has been reported under fluctuating light conditions (Bellafiore et al. 2005), implying that state transitions are beneficial for frequent changes in daily light levels. How is such a benefit in growth under fluctuating light achieved in WT compared to the *stn7* mutant? When plants are growing under low but fluctuating light conditions the importance to maintain strict homeostasis in the stroma is likely to become crucial. Indeed, strong variations in the stromal redox conditions in response to changed environment (Oswald et al. 2001; Piippo et al. 2006) are known to induce signalling cascades to the nucleus to initiate the stress response and/or acclimation processes. These signals possibly include thiol reductants or various ROS species in the stroma (Foyer and Noctur 2005). Such signaling occurs rapidly and changes in transcript levels are often seen already in the time scale of minutes. Here we report that in the *stn7* mutant the transient expression of various *HSP* genes was much stronger than in WT upon expose of plants to transient high light stress. HSPs are general stress proteins that contribute significantly to cell stress tolerance and are produced in response to many environmental stresses (e. g. heat, oxidative stress and high light stress) (Desikan et al. 2001; Kimura et al. 2003; Rossel et al. 2002; Takahashi et al. 2004). If the light conditions are soon likely to return to ''normal'', it would be tremendous wasting of energy to keep sending the signals and even responding to the signals by protein synthesis only to reverse the signal and degrade the newly synthesized proteins within a short time course. Therefore, it is conceivable that the short-term state transitions interact with long-term light acclimation. We see it highly likely that the beneficial growth of WT plants under fluctuating light as compared to *stn7* plants (Bellafiore et al. 2005) is due to a flexible control of the stromal redox state regulated by state transitions in WT thylakoids so as to avoid energy losses through meaningless signaling attempts, protein synthesis and their reversions. It is intriguing to note that the transition from state 2 to state 1, induced by an inhibition of the STN7 kinase upon accumulation of reducing equivalents in the stroma (Rintamäki

et al. 2000; Hou et al. 2003), appears to be a crucial process in successful plant acclimation to fluctuating, yet generally low light conditions.

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