Physiology

Light-harvesting complex B7 shifts the irradiance response of photosynthetic light-harvesting regulation in leaves of Arabidopsis thaliana

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The nuclear LHC7 gene is common in higher plants, encodes a transcript that is well expressed in a subset of leaf mesophyll cells, and is associated with a protein product that is homologous to pigment-binding complexes of the photosystem (PS) II peripheral antenna complex. We compared the physiological properties of wild type and LHC7-deficient leaves [DNA insertion, Arabidopsis thaliana (At) ecotype Columbia] in terms of pigment content, CO₂ exchange, in vivo transmittance at 810 nm, and chlorophyll fluorescence. The latter two techniques are functional indicators for PSI and PSII, respectively. Key features of the mutant phenotype were confirmed using antisense technology and a hemizygote of two independent AtLHC7 DNA insertion lines. Growth, leaf pigment composition, white light absorbance, and levels of AtLHCBI-6 were not significantly different in the mutant compared to wild type. Likewise, neither intrinsic PSI light capture efficiency nor partitioning of absorbed radiation to PSII was affected by the mutation. The absence of AtLHC7 is associated with lower rates of light-saturated photosynthesis and a diminished irradiance threshold for induction of photoprotective non-photochemical quenching. Overall, the pattern of change in light utilization parameters and plastocynol level indicated that loss of AtLHC7 expression led to slower Rubisco turnover characterized by pH-dependent balancing of electron transport to reduced carbon assimilation capacity (photosynthetic control). No effect of AtLHC7 genotype on xanthophyll de-epoxidation state was detected suggesting that factors in addition to lumenal pH influence zeaxanthin accumulation.

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Introduction

Chloroplast pigment-binding proteins of higher plant light-harvesting complexes (LHCs) capture radiant energy that drives the primary redox reactions of photosynthesis. The LHCA and LHCBI families comprise the peripheral antennae of Photosystems I and II (PSI and PSII), respectively (Green and Durnford, 1996). In the higher plant model Arabidopsis thaliana (At), approximately 30 nuclear LHC superfamily loci encode the LHCA and LHCBI proteins, early light-inducible proteins (ELIPs), ferrochelatase and psbS (Jansson, 1999). Currently, nine LHCA families have been identified based on comparisons of amino acid sequence similarity across species (i.e. LHCBI-9). In arabidopsis and other higher plants, the major LHCI proteins LHCBI, LHCBI, and LHCBI are encoded by multigene families while LHCBI, LHCBI, and LHCBI are encoded by single loci. The outermost PSI antenna proteins (LHCBI-3) normally exist as heterotrimers. The LHCBI-6 monomers connect the trimer complexes to the PSI core containing the photochemical reaction center (Dekker and Boekema, 2005; Allen et al., 2011).

Two novel arabidopsis chlorophyll (Chl) a/b-binding proteins, AtLHCB7 and AtLHCB8 (previously AtLHCB4.3) were inferred from a subset of “rarely expressed” LHC genes based on expression patterns across different tissues or laboratory conditions (Klimmek et al., 2006). Later, a new locus apparently unique to Physcomitrella patens was designated as LHCBI (Alboresi et al., 2008). Overall expression patterns were similar for AtLHCB7 and AtLHCB8 but differed substantially from the 10 abundant LHCs (AtLHCA1-4 and AtLHCB1-6). Association of these proteins with the peripheral

Abbreviations: A, antheraxanthin; ANOVA, analysis of variance; CA, complementary area; Chl, chlorophyll; Col, ecotype Columbia; Cyt, cytochrome; F₅₀, F₈₀, F₉₅, fluorescence yields: minimum, steady-state, maximum; FR, far red light; HL, high light; LE, ecotype Landsberg erecta; LHC, light-harvesting complex; LL, low light; LHCA, LHCB, light-harvesting complexes of photosystem I, II; ML, medium light; NPQ, non-photochemical quenching; p, probability; PAD, photon absorption density (μmol m⁻² s⁻¹); PFD, incident photon density (μmol m⁻² s⁻¹); PC, plastocyanin; PQH₂, plastoquinone (reduced); PSI, PSII, photosystem I, II; Photos, primary electron donor of PSI; Qₒ, Qₑ, photosystem II acceptor side quinone electron carriers; R°, coefficient of determination; (RT)-PCR, (reverse transcriptase)-polymerase chain reaction; SE, standard error of the mean; V, violaxanthin; WL, white light; WT, wild type; Z, zeaxanthin.

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light-harvesting complex of PSII was inferred by sequence homology (LHCBC7 is closest to LHCBC5) and low interspecies similarities (Klimmek et al., 2006). Characteristic LHCBC structural features such as pigment-binding sites, transmembrane helices, and a trimerization motif were conserved for a role at LHCBC7. It occurs widely in land plants but not algae consistent with a role in adaptation to a dynamic light environment (Klimmek et al., 2006; Alboresi et al., 2008).

Information on individual function among the structurally redundant LHCBC proteins has been sought using T-DNA and antisense technology. LHC-deficient plants invariably retained the ability to grow autotrophically. Nevertheless, co-suppression of AtLHCBC1/AtLHCBC2 and of loss of AtLHCBC6 modestly reduced the capacity to form the pH-dependent, reversibly phase (qE) of non-photochemical quenching (NPQ) (Andersson et al., 2003; Kovács et al., 2006; Bianchi et al., 2008). Although CO2 uptake rates were unaffected by loss of AtLHCBC1/AtLHCBC2 a significant decline in PSII photochemical efficiency [(Fm − F0)/Fm] for dark-adapted leaves indicated some destabilization of PSII macro-organization (Andersson et al., 2003). Antisense inhibition of AtLHCBC5 and AtLHC4 accumulation had no effect on qE and about 25% loss of qE, respectively (Andersson et al., 2001). On the other hand, photosynthetic rates were slightly lower for the AtLHCBC4-deficient line but were enhanced by suppression of AtLHCBC5 accumulation. Dark-adapted Fv/Fm values indicated some changes in organization of the light-harvesting antennae (Andersson et al., 2001). In contrast to the mild effects of LHC loss on growth under controlled conditions, prolonged exposure of these arabidopsis lines to natural conditions often resulted in reduced fitness (Andersson et al., 2003; Ganetege et al., 2004). Thus, slight structural variations cumulatively confer functional flexibility needed for robust growth in fluctuating light.

The role of AtLHCBC7 in photosynthetic light-harvesting has not heretofore been explored. Insertional mutagenesis often results in complete loss of gene function (O'Malley and Ecker, 2010). Alternative approaches based on antisense, RNAi, or chemically-induced point mutations can result in some accumulation of a functional product. We describe compositional and functional consequences of DNA insertion into the sole AtLHCBC7 locus (At1g76570).

Materials and methods

Plant material and growth conditions

Seed of Arabidopsis thaliana (L.) Heynh. lines SALK_018360C [ecotype Columbia (Col); Alonso et al., 2003] and WiscDSLox 506A05 (Col; Woody et al., 2007) were obtained from The Arabidopsis Biological Resource Center. A third line, GT20564 [ecotype Landsberg erecta (LE); Sundaresan et al., 1995], was obtained from Cold Spring Harbor Laboratory. Each line reportedly contained a unique DNA insertion into AtLHCBC7 (At1g76570). Plants were grown in a growth chamber in fertilized Super Fine Germinating Mix (Conrad Fafard, Agawam, MA) at an incident photon flux density (PFD) of 130 µmol quanta m⁻² s⁻¹ and a 16/8 h day/night regime and 23/20 °C. Light was supplied by a combination of Cool White fluorescent and incandescent lamps. Fully expanded rosette leaves were examined at 3–4 weeks after sowing.

Gas exchange and optical methods

The two-channel fast-response leaf gas exchange measurement system (Fast-Est, Tartu, Estonia) has been described (Laisk et al., 2002; Peterson, 2005). An excised leaf was enclosed in a temperature-controlled (leaf temperature of 22.8–23.2 °C), flow-through sandwich-type chamber and flushed with gas (380 µmol CO₂ mol⁻¹, 2.0% O₂, balance N₂) at 0.5 mmol s⁻¹. Distilled H₂O was provided to the petiole. Uptake of CO₂ was monitored with an infrared gas analyzer LI 6252 (Licor, Lincoln, NE, USA) and a micro-photometer detected transpiration. Calculation of dissolved CO₂ concentration at the carboxylation site (Ci, µM) considered stomatal and liquid phase diffusion resistances. The linear electron transport rate (Jc, see Laik and Loreto, 1996) associated with photosynthetic carbon metabolism was calculated as:

\[ J_{c} = 4(A + R_{k}) \frac{2K_{c}C_{c} + 2O_{c}}{2K_{c}C_{c} - O_{c}} \]

where A is the rate of net CO₂ assimilation (µmol m⁻² s⁻¹), Rk is Kreb cycle respiration in the light, Kc is the Rubisco CO₂/O₂ specificity factor (105 at 23 °C; Peterson and Havir, 2001), and Cc and O₂ are the carboxylation site dissolved CO₂ and O₂ concentrations (µM). At limiting irradiance Rk was assumed to equal the rate of CO₂ evolution measured in prior darkness. At higher irradiances the Rk observed previously in arabidopsis (0.106 µmol CO₂ m⁻² s⁻¹) was employed (Peterson and Havir, 2001). All light beams were directed to the adaxial leaf surface by a multi-branched fiber optic guide (Fast-Est, Tartu, Estonia). Actinic white light (WL) and fluorescence saturation pulses (10000 µmol quanta m⁻² s⁻¹ for 1.5 s) were provided by Schott KL 1500 tungsten halogen sources. A calibrated Licor Quantum Sensor (400–700 nm) mounted in the illumination apparatus continuously monitored the actinic PFD at the leaf surface. Far red light (FR, 50 µmol quanta m⁻² s⁻¹, 720 nm) was provided by a feedback-stabilized light-emitting diode source (Fast-Est, Tartu, Estonia). Sequence and execution of measuring procedures were standardized by use of a command textfile in conjunction with the control/acquisition software.

Chl fluorescence yield was measured with a PAM-101 equipped with an ED101 emitter-detector unit (H. Walz, Effeltrich, Germany). The quantum yield of PSII electron transport (φ₂) is based on the model of Genty et al. (1989):

\[ \phi_{2} = \frac{F_{m} - F_{m}}{F_{m}} \]

where

\[ qP = \frac{F_{m} - F_{s}}{F_{m} - F_{o}} \]

Note that fluorescence yields Fm and Fs correspond to qP=0 and qP=1, respectively, and Fm = Fm - Fo. The coefficient for partitioning of absorbed WL quanta to PSII (a₂) was measured at limiting irradiance as described by Peterson et al. (2001):

\[ a_{2} = \frac{F_{m}}{F_{m} - 1} \]

NDQ is expressed as the rate constant for H⁺-regulated de-excitation in PSII (Laik et al., 2002):

\[ NPQ = \frac{F_{m}}{F_{m} - F_{m}} - 1 \]

Fm is the “predawn” maximum yield measured after ≥12 h of darkness. Other details pertaining to signal processing have been described (Peterson et al., 2001; Peterson, 2005).

Changes in leaf transmittance at 810 nm were employed as a probe of PSII function using a single beam spectrophotometer FS810A (Fast-Est, Tartu, Estonia; Talts et al., 2007). The modulated (80 kHz) measuring beam was directed to a 1-cm² area at the center of the adaxial side of the test leaf. A photodetector facing the abaxial side registered transmission. Increased absorption of 810-nm radiation due to accumulation of P700⁺, oxidized plastocyanin (PC⁺), and reduced ferredoxin was isolated after compensation of the full signal voltage, amplified prior to processing by a 12-bit analog-to-digital converter, and displayed as a positive change. Electro-pneumatic shutters (flying time of 1.3 ms) enabled recording of rapid, irradiance-dependent changes in the 810-nm signal.

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A shortpass filter in the ED101 detector channel facilitated simultaneous recording of fluorescence and 810-nm transmission signals. Each test leaf (pre-darkened ≥12 h) was mounted in the chamber, followed by recording of predawn fluorescence yields (F₀ and Fₘₚ; Peterson et al., 2001). The leaf was then pre-illuminated at a PFD of 200 μmol quanta m⁻² s⁻¹ until steady state photosynthesis was attained. The stable CO₂ uptake rate; light-adapted fluorescence yields Fₛ, Fₚ, and F₀; and light-dark 810-nm transmittance changes (six-fold signal averaging) were recorded for successive WL test irradiances (Peterson, 2005). Measurement of F₀ included a 3-s exposure to FR to fully oxidize the PSI acceptor side as described by Peterson and Havir (2004). Total (400–700 nm) leaf absorbance for actinic WL (α) was measured with an integrating sphere using a KL 1500 tungsten halogen source (Peterson and Havir, 2001). The photon absorption density (PAD) was calculated from the incident photon flux density (PFD) as PAD = α × PFD.

Pigment analyses and immunoblots

Pigment separation by HPLC of 80% acetone extracts of leaf tissue has been described (Peterson and Havir, 2000). Immunoblots of psbS (Peterson and Havir, 2004) and LHCb proteins in leaf membranes were performed according to manufacturer (Agrisera) recommendations.

DNA and RNA manipulations

DNA was extracted from leaves according to Dellaporta (1993). RNA isolation was carried out using TRIzol (Invitrogen, Carlsbad, CA) and reverse transcription of RNA with a RCM-RACE Kit (Ambion, Austin, TX) according to manufacturer recommendations. Oligonucleotide primers AtLHCB7A (′5′ATGCCACGTGTTCAGAGAGCC 3′), AtLHCB7C (′5′GAACCATATCCCTGGAAG 3′), and P745 (′5′AACGTCGACATGGTTAATAGTGTTC3′) were used to genotype individual plants in a segregating population of WiscDsLox506A05 by PCR. Oligonucleotides AtLHCB7A, AtLHCB7E (′5′AATCTCAGATGTCTGCAAGCTGGCAATC3′) and Lb (′5′CTTTTAAATACCGTCTGGCAGACATC3′) were used to genotype plants in the CSHL GT20564 lines. Primers AtLHCB7A and AtLHCB7C were used to amplify a 408-bp AtLHC7B-derived cDNA fragment from reverse transcribed Col leaf RNA and cloned into pCR2.1-TOPO (Invitrogen, Grand Island, NY) to form plasmid pRh527. pRh527 was restricted with endonucleases KpnI and PsI to clone the AtLHC7B fragment in antisense orientation in binary plasmid pRh118 [composed of pCAMBIA1200 (Genbank# AF234292) and the 35S cassette from pFF19 (Timmermans et al., 1990)] to form plasmid pRh529. pRh529 was transformed into Agrobacterium tumefaciens GV2260 and used to transform Col by the floral dip method (Clough and Bent, 1998). Oligonucleotides AtLHCB7A and AtLHCB7C were used in reverse transcriptase-PCR (RT-PCR) analyses. The PCR parameters employed were: 94°C 3 min; 94°C 30 s, 55°C 30 s, 72°C 1 min) repeated 30× followed by 72°C 7 min.

Results

Molecular characterization of AtLHC7B-deficient lines

Locus At1g76570 contains six exons (Fig. 1A) encoding a unique protein designated as AtLHC7B by Klimmek et al. (2006). Three lines were reported to contain DNA insertions in exons of At1g76570. Lines WiscDsLox506A05 and GT20564 harbor T-DNA and “gene trap” insertions, respectively. Fig. 1B shows PCR results for homozygous wild type (WT) and mutant plants from a segregating population of WiscDsLox506A05. This mutant allele is designated AtLhcb7-1. Line GT20564, as obtained, was homozygous for the insertion in exon 3 (Fig. 1C) and designated AtLhcb7-2.

Fig. 1. Molecular analysis of the At1g76570 gene. (A) At1g76570 structure. Boxes and lines represent exon and intron sequences, respectively. Triangles correspond to putative T-DNA insertions. Gray triangles labeled WISC (line WiscDsLox506A05, insertion in exon 2) and CSHL (GT20564, insertion in exon 3) correspond to loci confirmed below. A reported insertion at the open triangle labeled SALK (SALK_018360C) in exon 1 was not confirmed (see text). Arrows a, c, e, p, and b denote the positions and orientations of oligonucleotide primers AtLHCB7A, AtLHCB7C, AtLHCB7E, P745 and Lb, respectively (see Materials and Methods). The DNA fragment sizes (base pairs, bp) generated by PCR for WT and insertion mutant chromosomes are indicated. A 410-bp DNA fragment is generated under RT-PCR treatment of At1g76570-specific mRNA transcripts (primers a and c); (B) Genotype analysis for the WISC locus (AtLHC7B-1). Lane 1 shows the 659-bp DNA band resulting from PCR treatment of genomic DNA from WT segregants while lanes 2–5 contain a 365-bp fragment derived from homozygous mutant transcripts. (C) Genotype analysis for the CSHL locus (AtLHC7B-2). Lane 1 shows the 1600-bp DNA band resulting from PCR treatment of genomic DNA from WT tissue while lanes 2–5 contain a 1100-bp fragment derived from homozygous mutant transcripts. (D) Expression analysis of WT Col and AtLhcb7-1 mutant tissue. Shown are results of RT-PCR treatment of mRNA from homozygous WT leaves (lanes 1–3) and homozygous mutant leaves (lanes 4–7) for At1g76570- and actin-specific products. (E) Expression analysis of WT ecotype Landsberg erecta (LE) and AtLhcb7-2 mutant tissue. Shown are results of RT-PCR treatment of mRNA from homozygous WT leaves (lane 1) and homozygous mutant leaves (lanes 2–5) for At1g76570- and actin-specific products.

Homozygous mutant plants are hereafter referred to by allele designations. Sequencing of DNA revealed that SALK_018360C does not contain an insertion in exon 1 (Fig. 1A) as reported by Alonso et al. (2003). Instead, a T-DNA insertion was found upstream of exon 1 in an orientation compatible with AtLHC7B expression. Analysis of At1g76570 mRNA by (reverse transcriptase-)polymerase chain reaction (RT-PCR) showed that homozygous WT leaves generated a 410-bp AtLHC7B-specific DNA fragment while no AtLHC7B transcript was observed for AtLhcb7-1 and AtLhcb7-2 plants (Fig. 1D and E). Thus, molecular genotypes correlate with AtLHC7B transcript accumulation confirming that AtLhcb7-1 and AtLhcb7-2 are true knockouts (null alleles) of At1g76570. Line WiscDsLox506A05 segregated WT siblings which served as ideal controls for physiological assessments. Since an isogenic WT strain could not be confirmed for AtLhcb7-2 we focus on the properties of AtLhcb7-1, an antisense strain prepared in Col, and a hemizygous knockout
obtained as an F1 cross of AtLhcb7-1 and AtLhcb7-2 (AtLhcb7-1/2).

AtLHC7-deficiency does not alter the contents of pigments or major PSII antenna proteins

Homozygous AtLhcb7-1 plants were not discernibly affected in appearance or growth compared to WT. Leaf pigment levels [10–30 replicates (N) per line] were not significantly different at the 5% probability level based on a T-test (i.e. p > 0.05). The overall average [±standard error (SE)] Chl a + b content was 195 ± 6 μmol m⁻² and the Chl a/b was 2.65 ± 0.04 mol mol⁻¹. Combined xanthophyll cycle [violaxanthin (V) + antheraxanthin (A) + zeaxanthin (Z)], lutein, neoxanthin, and β-carotene levels were 0.186 ± 0.006, 0.639 ± 0.010, 0.127 ± 0.001, and 0.305 ± 0.004 mol (mol Chl a)⁻¹, respectively. Consistent with the pigment data, respective mean leaf white light (WL) absorbances for WT and AtLhcb7-1 were 0.825 ± 0.012 (N = 7) and 0.810 ± 0.012 (N = 10) and not statistically different. Immunoblots of leaf membrane proteins from WT and AtLhcb7-1 were identical with respect to AtLHC1-6 and AtPsbS contents (not shown).

Contrasting light utilization properties of WT and AtLhcb7-1

Although identical in saturating light, the rise in NQO (Eq. (5)) in low light is shifted to a lower irradiance range for the mutant (Fig. 2). Two-way analysis of variance (ANOVA) indicated that the effect of genotype is significant (p = 0.025). We note that maximum fluorescence yields (Fm) was measured after 80–120 s at each irradiance level. Repeated measurements of NPQ at a nominal incident photon density (μmol m⁻² s⁻¹)(PFD) of 120 μmol m⁻² s⁻¹ (see legend to Fig. 2) were reproducible indicating an absence of latency for both lines. Although the familiar formula for NPQ reflects changes in Fm (PSII reaction centers closed), increased thermal dissipation also lowered emission when centers were open (minimum fluorescence yield: (F0)). Plots of Fm/F0 versus Fm/F0 (not shown) for WT and mutant were linear (slope = 1.37 ± 0.04) yet indistinguishable consistent with a common antenna-based quenching mechanism (Dau, 1994; see also Peterson and Havir, 2004).

The redox state of the initial quinone electron acceptor in PSII (QA) is given by qP (Eq. (3)). Fig. 3 shows the irradiance responses of qP, the quantum yield of linear electron transport supporting carbon metabolism (ϕe = Jc/PSII, Eq. (1)), and PSII photochemical yield (ϕ2, Eq. (2)). Nearly identical results were obtained for WT and mutant in limiting light. However, a shift in downregulation of light use efficiency to a lower irradiance range in AtLhcb7-1 occurred as light became progressively less limiting. Two-way ANOVA indicated a highly significant effect of genotype for each of these parameters (p < 0.001). Plots of ϕe (Fig. 3B) versus ϕ2 (Fig. 3C) were quasi-linear yet undistinguishable for WT and mutant (not shown). Table 1 summarizes the properties of WT and AtLhcb7-1 using a measuring protocol targeted to limiting (low light (LL)), intermediate (medium light (ML)), and saturating (high light (HL)) light. There was no significant effect of AtLHCB7 genotype on PSII “predawn” quantum yield (Fv/Fm) or the fraction of absorbed WL quanta partitioned to PSII (a2, Eq. (4)). Although mean stomatal resistance to CO₂ diffusion (Rw) was slightly higher in AtLhcb7-1, this effect was statistically insignificant (p = 0.05). Conversely, effects of AtLHCB7 expression on Jc, NPQ, qP, and ϕ2 were significant indicating higher PSII photochemical yield for WT. Higher PSII excitation capture efficiency (Fv/Fm, Eq. (2)) for WT is consistent with the lower NPQ values observed.

Assessment of PSI function can corroborate interpretations based on Chl fluorescence. Increases in in vivo absorbtion at 810 nm are due predominantly to accumulation of oxidized forms

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of the PSI primary donor (P700⁺) and the mobile PSI electron donor (plastoquinin (PC⁺)) (Harbinson and Woodward, 1987). Their steady state redox levels in the light reflect a dynamic balance among P700 photo-oxidation, electron donation from PSII, and cyclic electron flow (Harbinson et al., 1990; Laisk et al., 2010). Consequently, a positive albeit frequently nonlinear relationship between φ₂ and the photo-oxidizable fraction of the 810-nm signal, φ₁, is observed. The steady state 810-nm signal in actinic light is recorded as W₁. A 20-ms saturating flash (10000 μmol quanta m⁻² s⁻¹) simultaneously reduces the PQ pool and photo-oxidizes PSI centers possessing acceptor side electron vacancies (Peterson, 2005). Following the flash, PQH₂ reduces the PSI donor side establishing the dark baseline. Thus,

φ₁ = \frac{W₂ - W₁}{A810_{max}} \tag{6}

where A810\text{max} is the full signal obtained by superimposing a 20-ms saturating WL flash on a far red light (FR) background illumination (50 μmol quanta m⁻² s⁻¹, Talts et al., 2007). Table 1 shows that φ₁ was lower in AtLhcb7-1 compared to WT leaves to a relative extent similar to the effect on φ₂.

Confirmation of the AtLhcb7-1 phenotype

The pattern of enhancement of NPQ and declines in qP, φ₂, and φ₁ as irradiance increased suggested reduced assimilatory capacity in AtLhcb7-1 relative to WT based on responses to manipulation of atmospheric CO₂ level in normal leaves (Peterson, 2005). Since this was unexpected for a PSI antenna protein mutant, we considered the possibility that a cryptic mutation linked to the AtLhcb7 locus in WiscDsox506A05 inhibited Calvin cycle function. An isogenic WT control for AtLhcb7-2 (Fig. 1) was unavailable. Thus, we examined; (1) WT Col plants carrying an AtLhcb7 antisense transgene and (2) a hemizygous AtLhcb7 knockout line obtained by crossing AtLhcb7-1 and AtLhcb7-2. The effect of a hidden mutation in AtLhcb7-1 would be masked by the normal allele donated by AtLhcb7-2 in the hemizygote (AtLhcb7-1/2). Effects of light level on several parameters for transgenic and AtLhcb7-1/2 plants as well as WT and AtLhcb7-1 are shown in Table 2. To enhance sensitivity, each entry is a ratio of measurements at two light levels. Parameters other than NPQ are normalized relative to LL (minimal downregulation of quantum yield). Since NPQ is maximal in HL (Fig. 2), measurements were normalized to HL. Overall, the pattern of change observed for WT versus AtLhcb7-1 was also observed for WT versus hemizygous mutant and transgenic plants ruling out interference by a second mutation. Exceptions were \( F_v/F_m \) for the antisense plants at both irradiance ratios and AtLhcb7-1 and AtLhcb7-1/2 for the HL/LL comparisons. Since NPQ suppresses both \( F_v \) and \( F_o \) (Fig. 3), \( F_v/F_m \) is less sensitive to changes in thermal dissipation.

Loss of AtLhcb7 expression enhances PQH₂ accumulation

Oxidation of PQH₂ is a control point in the PSI–PSII electron transport sequence \( \text{H}_2\text{O} \rightarrow \text{Q}_{A} \rightarrow \text{Q}_{B} \rightarrow \text{PQ} \rightarrow \text{Cyt} \rightarrow \text{PC} \rightarrow \text{P700} \) (Foyer et al., 1990). Transient changes in the \textit{in vivo} 810-nm signal under a FR background following cessation of actinic WL were used to quantify electron donor pools upstream from PSI, primarily PQH₂ (Asada et al., 1992, 1993). Fig. 4 shows that the drop in excitation of PSI caused by interruption of actinic WL (panels B, C, and inset) resulted in rapid reduction of the PSI donor side followed by a slower FR-dependent re-oxidation as equivalents from PQH₂ were transferred via P700 to the acceptor side of PSI. Integration of the signal change time course (complementary area, CA) constitutes a relative approximation of the size of the electron reservoir upstream of PSI under the steady state WL+FR. Leaves of WT showed a >3-fold increase in CA in HL (Fig. 4C) compared to ML (Fig. 4B) consistent with higher feedback downregulation of
Table 2
Normalized responses to irradiance relative to WT for knockout strains (AtHBch7-1, AtHBch7-1/2) and plants possessing an antisense construct against AtHBCh7.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Irradiance ratio</th>
<th>WT (Col) N=12</th>
<th>AtHBch7-1 N=11</th>
<th>Antisense AtHBch7 N=15</th>
<th>FI cross AtHBch7-1/2 N=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \phi_i )</td>
<td>ML/LL</td>
<td>0.477 ± 0.028</td>
<td>0.377 ± 0.034</td>
<td>0.379 ± 0.015</td>
<td>0.350 ± 0.010</td>
</tr>
<tr>
<td></td>
<td>HL/LL</td>
<td>0.132 ± 0.007</td>
<td>0.109 ± 0.008</td>
<td>0.104 ± 0.005</td>
<td>0.098 ± 0.004</td>
</tr>
<tr>
<td>( qP )</td>
<td>ML/LL</td>
<td>0.708 ± 0.018</td>
<td>0.604 ± 0.030</td>
<td>0.648 ± 0.019</td>
<td>0.563 ± 0.019</td>
</tr>
<tr>
<td></td>
<td>HL/LL</td>
<td>0.220 ± 0.005</td>
<td>0.190 ± 0.011</td>
<td>0.189 ± 0.011</td>
<td>0.168 ± 0.013</td>
</tr>
<tr>
<td>( \phi_i )</td>
<td>ML/LL</td>
<td>0.620 ± 0.018</td>
<td>0.514 ± 0.028</td>
<td>0.565 ± 0.020</td>
<td>0.468 ± 0.023</td>
</tr>
<tr>
<td></td>
<td>HL/LL</td>
<td>0.181 ± 0.005</td>
<td>0.154 ± 0.008</td>
<td>0.157 ± 0.009</td>
<td>0.135 ± 0.011</td>
</tr>
<tr>
<td>( \phi_i )</td>
<td>ML/LL</td>
<td>0.760 ± 0.015</td>
<td>0.644 ± 0.010</td>
<td>0.675 ± 0.025</td>
<td>0.594 ± 0.018</td>
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<tr>
<td></td>
<td>HL/LL</td>
<td>0.257 ± 0.007</td>
<td>0.231 ± 0.009</td>
<td>0.228 ± 0.010</td>
<td>0.225 ± 0.009</td>
</tr>
<tr>
<td>( F_o/F_m )</td>
<td>ML/LL</td>
<td>0.875 ± 0.006</td>
<td>0.849 ± 0.007</td>
<td>0.870 ± 0.009</td>
<td>0.829 ± 0.018</td>
</tr>
<tr>
<td></td>
<td>HL/LL</td>
<td>0.820 ± 0.007</td>
<td>0.809 ± 0.012</td>
<td>0.832 ± 0.007</td>
<td>0.802 ± 0.018</td>
</tr>
<tr>
<td>NPQ</td>
<td>ML/LL</td>
<td>0.006 ± 0.013</td>
<td>0.055 ± 0.016</td>
<td>0.049 ± 0.009</td>
<td>0.044 ± 0.018</td>
</tr>
<tr>
<td></td>
<td>HL/LL</td>
<td>0.601 ± 0.036</td>
<td>0.722 ± 0.039</td>
<td>0.689 ± 0.026</td>
<td>0.717 ± 0.037</td>
</tr>
</tbody>
</table>

The first five parameters show the ratio of the value obtained in ML or HL to that obtained in LL. NPQ values are normalized to HL. Means for knockout and antisense plants are statistically compared to WT using a T-test. Asterisks are defined in Table 1. See text and Table 1 for additional information.

light utilization at the saturating HL level. No reduction transient was detected in LL (Fig. 4A) confirming a lack of PQH2 accumulation when PSII turnover limits the linear electronic transport rate. Most important, average CA values for AtHBch7-1 were 66% and 24% larger than WT in ML and HL, respectively, consistent with enhanced PQH2 accumulation in the mutant. We note also a detectably slower rise in the 810-nm signal for the mutant consistent with restricted oxidation of NADPH downstream from PSI (Fig. 5).

The transients of Fig. 4 are subject to minor distortion for two reasons. First, due to differences in the extinction coefficients for PC* and P700* and the midpoint redox potentials of these carriers the incremental change in the 810-nm signal per incremental change in reduction of the PSI primary donors is not constant (Oja et al., 2003). This ratio increases with the magnitude of the 810-nm signal. Secondly, return of electrons from the PSI acceptor side to PC* and P700* via a cyclic pathway would falsely inflate the CA values (Asada et al., 1993). Indeed, FR-driven PSI cyclic electron flow is favored with increasing oxidation of PC and P700 (Talts et al., 2007; Laik et al., 2010). Since, on average, the 810-nm signal is slightly (6–7%) higher for WT in ML and HL compared to mutant leaves over the 3-s interval used for assessment of CA we conclude that overestimation of CA is greater for WT. Consideration of these effects would magnify the increase in CA for AtHBch7-1 relative to WT. Lastly, the steady state signal attained after 2 s of FR (Fig. 4A) was essentially identical for WT (77.0% ± 0.5) and mutant (77.7% ± 0.4) suggesting no difference between these lines in FR-driven cyclic electron flow.

No effect of AtHBCh7 deficiency on V de-epoxidation

The pH-dependent conversion of V to Z by V de-epoxidase in the thylakoid lumen is associated with light-dependent formation of NPQ and the protonmotive force for ATP formation (Demming-Adams, 1990; Hager and Holocher, 1994; Avenson et al., 2004). Fig. 5 indicates no enhancement of V-de-epoxidase in AtHBch7-1 compared to WT. Despite incomplete reversal of A and Z accumulation in the dark, a 3-fold increase in de-epoxidation was observed saturating at a PFD of 700 μmol quanta m−2 s−1. Two-way ANOVA confirmed that the effect of genotype was not statistically significant (p = 0.39).

Discussion

Although challenging to characterize, weak phenotypes such as AtHBCh7-deficiency offer a rare advantage for probing the biochemical subtleties of photosynthetic regulation. Specifically, our assessment of AtHBCh7 function is unlikely to be confounded by gross effects on PSII integrity. We observed no effect on PSII absorption cross section (\( \alpha_2 \)), \( F_o/F_m \), and absorbance of WL. The apparent lack of effect of AtHBCh7-deficiency on pigment levels or compensatory changes in AtHBChB-1 content could be related to the limited expression of AtHBCh7. The “rarely expressed” genes AtE-CA6, AtHBCh7, and AtHBChB (Klimmek et al., 2006) were found to be well expressed in a subset of cells in various tissue loci (Sawchuk et al., 2008). Expression of AtHBCh7 occurred exclusively in mesophyll cells near the adaxial surface of the leaf. The normally higher expression levels in these cells led Sawchuk et al. to suggest that AtHBCh7 has a photoprotective role. On the contrary, our results indicate that maximal NPQ is unaffected by AtHBCh7 content and NPQ induction is shifted to a higher irradiance range in WT leaves. By contrast, V de-epoxidase, psbS, or lycopene ε-cyclase level does affect maximal NPQ capacity (Müller et al., 2001). Loss of AtHBCh7 expression is associated with depressed rates of electron transport (\( J_o \)), higher PQH2 levels (CA), and lower open PSII trap density (\( qP \)) and PSI/PSII quantum yields (\( \phi_i \), \( \phi_e \)) characteristic of response to a downstream limitation in assimilatory capacity (Genty et al., 1989; Harbinson et al., 1990; Peterson, 2005). This altered regulatory pattern is maintained in an AtHBCh7 antisense line and in the AtHBch7-1/2 hemizygote (Table 2) ruling out interference by a cryptic, second mutation affecting carbon fixation.

A conceptual organizing principle of photosynthetic regulation is that Rubisco activity is coordinated with PSII, PSI, and cytochrome (Cyt) b6/ function; broadly referred to as “photosynthetic control” (Genty et al., 1989; Foyer et al., 1990; Eichelmann et al., 2009). Manifestations of this control are NPQ: accumulation of QA*, PQH2, PC*, and P700*; closure of PSI centers by acceptor side reduction; and resistance to electron transfer from PQH2 to Cyt f. Accumulation of H+ ions in the thylakoid lumen orchestrates these responses. The dominant mε phase of NPQ is considered to follow protonation of sites in the PSII antenna inducing a conformational change aided by A and Z accumulation (Krause, 1973; Hager and Holocher, 1994; Horton et al., 1996; Gilmore, 1997; Avenson et al., 2004; Ruban et al., 2001, 2007). Co-transport of electrons and H+ during PQH2 oxidation is opposed by lumenal H+ leading to higher reduction of QA (Siggel, 1974; Harbinson and Hedley, 1989). The luminal pH is maintained at a moderate 5.8–6.5, permitting ATP synthesis and regulation while avoiding H+–induced damage (Kramer et al., 1999; Zaks et al., 2012). Thus, effects of loss of AtHBCh7 expression are consistent with imposition of photosynthetic control mediated by luminal pH. An intriguing possibility is that the antenna component AtHBCh7 could be the postulated messenger linking photochemical yield and Rubisco Activase function which controls Rubisco active site turnover (Eichelmann et al., 2009).

The significant shifts in light-dependent induction of NPQ (Fig. 2) and \( qP \) (Fig. 3) contrast with phenotypes selected for altered thermal dissipation capacity. Arabidopsis mutants deficient in NPQ such as
Fortuitously, the two distinct interpretations of the AtLHC7-deficient phenotype introduced here can be resolved. From the perspective of control of interphotosystem electron transport, upstream limitation (PSII function) should lower, while downstream limitation (Rubisco turnover) should raise, the PQH2 level. Thus, it is sufficient to ascertain whether PQH2 is less or more abundant in the mutant compared to WT under the same conditions. Analysis of changes in the 810-nm absorptance signal from PSII is best by contributions from multiple chromophores and coexistence of linear and cyclic electron flows (Oja et al., 2003; Talts et al., 2007; Laik et al., 2010). These factors were considered in our analyses of CA (Fig. 4). We conclude that the PQH2 pool is larger for the mutant when photons are present in excess confirming the downstream limitation alternative for electron transport rate.

Although higher levels of PQH2 for AtLhc7-1 are consistent with a lower luminal pH compared to WT this did not result in higher Z levels in the mutant as would be expected based on simple pH control of V de-epoxidase. Lower V de-epoxidase correlated with impaired transthylakoid ΔpH formation in an AtLHC6 knockout (Bianchi et al., 2008). On the other hand, several studies suggest that this pH-control model is insufficient. Differential effects on the relative activities of V de-epoxidase and Z epoxidase can result in shifts in the extent of accumulation of Z in response to transthy- lakoid ΔpH (Zhang et al., 2011). Using reflectance measurements to monitor Z levels in vivo, Peguero-Pina et al. (2013) described a complex relationship between NPQ and Z in Quercus coccifera. Three Z pools were identified and postulated to have different roles in photoprotection. In this study, the effect of a lower luminal pH in AtLhc7-1 may be offset by lower levels of ascorbate, a substrate for the V de-epoxidase reaction (Hager, 1965). Lower rates of linear electron transport in the mutant could lead to competition for NADPH between 3-phosphoglyceric acid reduction and conversion of monodehydroascorbate to ascorbate (Müller-Moulé et al., 2002).

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**References**


