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The expression of phototropins in *Arabidopsis* leaves: developmental and light regulation

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Abstract

Phototropins are blue light receptors, which play different roles during plant development. Two phototropins of *Arabidopsis thaliana*, phot1 and phot2, have strongly overlapping functions. In seedlings, both photoreceptors are responsible for phototropism. In mature leaves they redundantly regulate leaf shape, stomatal opening, and the accumulation of chloroplasts, whereas phototropin2 alone controls chloroplast avoidance response. Light not only activates phototropins, but also affects the level of their expression. In *Arabidopsis* seedlings, *PHOT1* is downregulated and *PHOT2* is upregulated by light. Since data on transcription levels of phototropins in mature *Arabidopsis* leaves is scarce, a comprehensive real-time PCR study of *PHOT1* and *PHOT2* expression during development was performed, from seedlings to senescing leaves. So far, neither the phototropin expression nor its modulation by light have been investigated during senescence. The results show that the general regulation pattern remains conserved during *Arabidopsis* lifecycle, whereas the level of transcripts fluctuates over time, pointing to the significance of the light control for functioning of phototropins. The second part of the study determined the influence of photosynthesis-derived signals and photoreceptor-activated transduction pathways on phototropin mRNA levels. The effects of blue and red light were examined using *Arabidopsis* mutant lines deficient in photoreceptors. The results reveal a complex network of interactions between these receptors in the regulation of phototropin transcription profiles. Cryptochrome1 and phytochromeB appear to be main photoreceptors involved in the regulation of *PHOT1* transcript accumulation. The expression of *PHOT2* is dependent on both cryptochromes and phytochromeA.

Key words: *Arabidopsis thaliana*, blue light, gene expression, mature leaves, photoreceptors, phototropin1, phototropin2, white light.

Introduction

Light is an essential element in the functioning of plants. It not only enables life and growth via photosynthesis, but also plays an important regulatory role in plant development. Four main families of photoreceptors allow plants to perceive light: red/far-red-absorbing phytochromes, blue/UVA-absorbing cryptochromes, Zeitlupe family proteins, and phototropins. The first three families control durable circadian rhythms, long-lasting growth, and developmental processes, while the latter one provides a fine-tuning mechanism by controlling rapid responses and transient movements.

Phytochromes and cryptochromes form a group of nuclear localized photoreceptors which control gene expression. In *Arabidopsis thaliana* the phytochrome family consists of five members and is divided into two types. *PHYA* is a type 1 'light labile' phytochrome. Type 2 'light stabile' phytochromes are represented by *PHYB*, *PHYC*, *PHYD*, and *PHYE* (Sharrock and Clack 2002). A characteristic photoconversion from a red-absorbing to a far-red-absorbing form of phytochromes is mediated by their chromophore, a covalently attached linear tetrapyrrole (Rockwell *et al.*, 2006).

The cryptochrome action is triggered by blue and UVA light perceived by flavin adenine dinucleotide and methenyltetrahydrofolate chromophores (Lin *et al.*, 1995; Malhotra *et al.*, 1995). Three plant cryptochrome genes have been found in the *Arabidopsis* genome: *CRY1*, *CRY2*, and a member of the *CRY DASH* family, *CRY3* (Li and Yang, 2007). Phytochromes are responsible for germination, shade avoidance response, and, together with cryptochromes, for de-etiolation, circadian entrainment, and flowering (for review, see Jiao *et al.*, 2007). The functional overlap of these two groups of photoreceptors in the control of plant development is attributable to similar forms of transcriptome modulation (Ma *et al.*, 2001; Tepperman *et al.*, 2001; Jiao *et al.*, 2003) and signalling pathways (Jiao *et al.*, 2007). In seedlings, even the overall pattern of genome expression remains similar regardless of whether the light treatment is white, far-red, red, or blue (Ma *et al.*, 2001).

The latter family of photoreceptors involved in the modulation of the circadian clock and flowering contains a group of three related genes: *ZTL* encoding Zeitlupe, *FKF1* encoding flavin-binding Kelch F-box1, and *LKP2* encoding LOV Kelch Protein2. Each of these proteins contains a LOV domain with flavin mononucleotide as a chromophore at the N-terminus and an F-box with six Kelch repeats at the C-terminus. The C-terminal domain mediates the light-dependent protein degradation via the proteasome pathway (Demarsy and Fankhauser, 2009).

The fourth family of photoreceptors – the phototropins – form a unique group. These blue-light-sensitive receptors consist of two parts: a C-terminal serine-threonine kinase and two LOV domains which bind flavin mononucleotide as chromophores at the N-terminus (Christie, 2007). *A. thaliana* has two phototropin genes, *PHOT1* (Liscum and Briggs, 1995) and *PHOT2* (Jarillo *et al.*, 2001; Kagawa *et al.*, 2001), which have highly redundant functions. In seedlings, phot1 mediates the inhibition of hypocotyl growth (Folta and Spalding, 2001) and both receptors are responsible for controlling phototropic curvature (Sakai *et al.*, 2001). In adult plants, their role broadens to optimize photosynthesis by acting in new versatile processes. Both phototropins regulate leaf expansion, stomatal opening (Kinoshita *et al.*, 2001), and chloroplast accumulation, whereas only phot2 controls the chloroplast avoidance response (Jarillo *et al.*, 2001; Kagawa *et al.*, 2001; Sakai *et al.*, 2001). Although cryptochromes are believed to play a principal role in regulating blue-light gene expression (Jiao *et al.*, 2003; Ohgishi *et al.*, 2004), several genes seem to be controlled also by phototropin1 in *Arabidopsis* (Chen *et al.*, 2008; Lehmann *et al.*, 2010) and by *Chlamydomonas* phototropin (Im *et al.*, 2006). Moreover, *Arabidopsis* phototropin1 promotes the destabilization of *Lhcb* and *rbcL* mRNAs mediated by high-fluence blue light (Folta and Kaufman, 2003).

Changes in plant transcriptome in response to light are triggered not only by photoreceptors, but also by retrograde signalling from the plastids, which reflects their energy state. Among various signals such as chloroplast metabolites, reactive oxygen species and protein synthesis, the leading role in the regulation of nuclear gene transcription is attributed to the redox state of the plastids (Kleine *et al.*, 2009; Pfannschmidt *et al.*, 2009). In *Arabidopsis*, the redox state of photosystem I has a stronger impact on short-term nuclear gene expression than that of the plastoquinone pool (Piippo *et al.*, 2006).

Gene expression profiles differ considerably in various organs (Ma *et al.*, 2005). Studies of phototropin mRNA expression profiles in *Arabidopsis* subjected to light treatment were performed only for etiolated seedlings, while little is known of what happens in green plants during their maturation. The amount of *PHOT2* is significantly higher in mature leaves than in other organs (Jarillo *et al.*, 2001; Kagawa *et al.*, 2001). In 2-day-old etiolated *Arabidopsis* seedlings, the expression of *PHOT1* diminishes under blue light (Kang *et al.*, 2008), whereas the expression of *PHOT2* is enhanced by red light in 4-day-old etiolated seedlings (Kagawa *et al.*, 2001), by UVA in 6-day-old etiolated seedlings (Jarillo *et al.*, 2001) and blue light in both 4- and 6-day-etiolated seedlings (Jarillo *et al.*, 2001; Kagawa *et al.*, 2001). Similarly, studies of phototropin rice homologues *OsPHOT1* and *OsPHOT2* show higher transcript levels in mature leaves than in seedlings and other plant organs including shoots, roots, and flowers. In addition, the *OsPHOT1* gene is downregulated in coleoptiles under white light, whereas the *OsPHOT2* gene is upregulated in coleoptiles and leaves (Jain *et al.*, 2007). On the other hand, two homologues of phototropin1 are differentially regulated by light in pea seedlings: the *PsPHOT1A* gene is upregulated, while the *PsPHOT1B* gene is downregulated (Elliot *et al.*, 2004). To fully elucidate the light and developmental relationships in phototropin expression in *Arabidopsis*, a comprehensive real-time PCR analysis was performed. A set of photoreceptor mutants and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of photosynthesis, were used to more thoroughly understand the effects of blue and red light on phototropin mRNA levels.

Materials and methods

Plants and plant growth conditions

A. thaliana wild-type Columbia and Landsberg *erecta* were obtained from Nottingham Arabidopsis Stock Centre (Nottingham, UK). Seeds of blue light photoreceptors mutants were the kind gift of J. Jarillo, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain (*phot1phot2*), Anthony R. Cashmore, Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, USA (*phot1*, *phot2*), and Chentao Lin, University of Illinois, Chicago, USA (*cry1*, *cry2*, *cry1cry2*). Seeds of phytochrome mutants were purchased from the Nottingham Arabidopsis Stock Centre (*phyA*, *phyB*, *phyAphyB*). Phytochrome mutants were Landsberg *erecta* background; cryptochrome and phototropin mutants were background Columbia. Plants were grown in a growth chamber (Sanyo MLR 350H, Japan) with 10/14 light/dark cycle at 23 °C, 80% relative humidity, and illumination by fluorescent lamps (FL40SS.W/37, Sanyo) with a photosynthetic photon flux density of 100 µmol m^{-2} s^{-1}. For the experiment with 1-week-old seedlings, the seeds were surface sterilized, sown on B5 medium with 0.7% agar and left etiolated or grown in a standard photoperiod in the growth chamber (MLR 350H, Sanyo).

Light treatments

To determine the light regulation of phototropin expression during growth, whole plants (1-, 2-, or 3-week-old) or only fifth and sixth rosette leaves (from 4-, 5-, 6-, and 7-week-old plants) were collected. Plants were dark-adapted for 16 h. Seedlings were exposed to light of 100 μmol m^{-2} s^{-1} in the growth chamber. Older plants were illuminated as seedlings, except for one leaf, which was covered with black paper and kept in darkness. After 3 h of light treatment, the exposed and dark-adapted leaves/seedlings were immediately frozen in liquid nitrogen.

To examine the role of photoreceptors in light regulation of phototropin mRNA, 5-week-old mutant plants were dark-adapted for 16 h. They were irradiated with blue or red light of 40 μmol m^{-2} s^{-1} for 3 h. The blue and red light were obtained from LEDs (ELEX-5AA0-D00, Edison Opto, Taiwan) using plastic foil filters. The respective maxima were 457 ± 10 nm and 627 ± 20 nm. Thereafter, dark-adapted (control) and irradiated leaves (pooled from at least ten different plants) were frozen in liquid nitrogen. It should be stressed that all *Arabidopsis* lines examined were grown concomitantly and illuminated at the same time of day.

For senescence studies, a model of plants growing in a standard photoperiod with individually darkened leaves was applied (Weaver and Amasino, 2001). Senescence was induced in 5-week-old plants and samples were collected for 5 days. Each day, at the same time, a leaf wrapped in black paper was either illumined with white light for 3 h (Philips Master TL-D 36W/840, Osram L 36W/77 FluorA, Germany, 120 μmol m^{-2} s^{-1}) or left untreated and afterwards frozen in liquid nitrogen.

DCMU treatment and chlorophyll fluorescence measurements

To inhibit photosynthesis, leaves of 5-week-old plants were sprayed with 200 μM DCMU (Diuron, Sigma-Aldrich) in 0.4% DMSO solution or with 0.4% DMSO alone as control, covered with plastic foil and kept in darkness overnight. To investigate the level of photosynthesis inhibition, the quantum yield of energy conversion in photosystem II was evaluated in whole plants. Measurements of chlorophyll fluorescence were carried out with an Open FluorCam FC 800-O/1010 imaging fluorometer (Photon Systems Instruments, Czech Republic). First basal fluorescence (F_0) was collected for 5 s in darkness, then fluorescence (F_m) in the presence of red actinic light (150 μmol m^{-2} s^{-1}) was measured for 10 s. The mean quantum efficiencies of photosystem II were determined for each dark-adapted leaf. Leaves treated with DCMU in DMSO or DMSO alone were either collected directly after the measurement of chlorophyll fluorescence and used as a dark control or they were harvested after determining the chlorophyll fluorescence and 3 h of irradiation with white light. All of the collected leaves were frozen immediately in liquid nitrogen.

Steady-state RNA level analysis

RNA from seedlings and photoreceptor mutants was isolated with a Spectrum Plant Total Kit (Sigma-Aldrich) and digested by DNaseI (Fermentas) during purification on the column. RNA from older plants and leaves (for experiments on the influence of age and senescence) was isolated using TRI Reagent (BioChemika), following procedures recommended by the manufacturer. The RNA concentration was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). RNA integrity was checked by electrophoresis on 2% agarose gel. Reverse transcription was prepared with the RevertAid M-MuLV Reverse Transcriptase Kit (Fermentas) using random hexamer primers. Real time reverse-transcription PCR was performed with SYBR Green JumpStart Taq ReadyMix (Sigma Aldrich) and a thermal cycler (Rotor-Gene 6000, Corbett Research, Australia). cDNA corresponding to 50 ng of RNA was used in a single reaction and all reactions were run in duplicates. The primer sequences for PHOT1 and PHOT2 and the reference genes *UBC*, *SAND*, *PDF2*, *EF1α*, based on Czechowski *et al.* (2005), are listed in Table 1. *PHOT1* and *PHOT2* primers were designed to flank introns. PCR conditions were as follows: 10 min at 95 °C and 40 cycles of 15 s at 95 °C, 15 s at 51/56 °C (depending on the primer set: 51 °C for reference genes, 56 °C for phototropins), and 20 s at 72 °C. The specificity of the obtained products was verified on a dissociation curve at the end of each run and by 2% agarose gel electrophoresis. The relative expression of each gene in a sample was determined using the mean value of C_t for dark-adapted wild-type leaves in a given run as a reference. For developmental experiments, the mean value of C_t for 4-week-old dark-adapted leaves was utilized. Normalization of the phototropin expression level was performed using normalization factors calculated by geNorm v3.4 (Vandesompele *et al.*, 2002). Each point represents the mean of at least three repetitions. The data from the photoreceptor mutant experiment represent at least three technical replicates. For statistical analysis, unpaired t-test with Welch correction was performed using GraphPad InStat software.

Results

The light transcription pattern of phototropins remains conserved in Arabidopsis *during the growth period*

To elucidate the effect of light on phototropin transcription during *Arabidopsis* development, whole plants were illuminated in the growth chamber for 3 h with white light of 100 μmol m^{-2} s^{-1}, and whole seedlings or leaves of older plants were collected respectively. Apart from week 1, the *PHOT1* transcript was slightly downregulated by light during the whole period of growth of *Arabidopsis* (Fig. 1A). In this experimental system, the light regulation of the *PHOT1* transcript observed in 1-week-old plants was negligible both in photomorphogenic and skotomorphogenic seedlings, but the *PHOT1* mRNA level was twice as high in the skotomorphogenic ones. For light-grown plants, the dark levels of *PHOT1* remained low in seedlings at very early stages of development (week 1), while in week 2 of growth a 3-fold rise in *PHOT1* expression was observed. The dark level of *PHOT1* transcript increased till week 7 of growth. The light downregulation of *PHOT1* mRNA was a rather weak effect in the conditions of the experiment, but *PHOT1* transcript decreased significantly in weeks 3 and 4.

Table 1. Sequences of primers used in the study

Primer	Sequence (5′–3′)
PHOT1F	CACTGATCCTAGGCTTCCCG
PHOT1R	GTGGTTAGATCAGTCTCTGGACC
PHOT2F	GCTACCACTCTTGAGCGCATAGAG
PHOT2R	CCTCGCGTGAATACTCTGTC
SANDF	AACTCTATGCAGCATTTGATCCACT
SANDR	TGATTGCATATCTTTATCGCCATC
PDF2F	TAACGTGGCCAAAATGATGC
PDF2R	GTTCTCCACAACCGCTTGGT
UBCF	CTGCGACTCAGGGAATCTTCTAA
UBCR	TTGTGCCATTGAATTGAACCC
EF1αF	TGAGCACGCTCTTCTTGCTTTCA
EF1αR	GGTGGTGGCATCCATCTTGTTACA

As stated earlier for seedlings, the *PHOT2* transcript was upregulated by light during the whole period of growth of *Arabidopsis* (Fig. 1B). Similar to the *PHOT1* transcript, *PHOT2* mRNA levels in light-treated plants showed a tendency to increase during the development of *Arabidopsis*. In darkness, the *PHOT2* expression in 1-week-old etiolated seedlings was about 5-fold lower than in seedlings grown under standard photoperiod and dark-adapted for 16 h only. After week 2 of growth, the amount of *PHOT2* transcript in the dark increased twice and remained at this level for the rest of the examined developmental period. The increase in the expression of *PHOT2* after light treatment was about 3-fold starting from week 2 of growth. It should be noted that this effect was stronger in seedlings, especially in the etiolated ones, where a 10-fold increase in the *PHOT2* mRNA level was observed.

Phototropin transcript regulation is conserved in senescing leaves, but the level of mRNA changes over time

To determine what occurs at the end of *Arabidopsis* development, Weaver and Amasino's model (2001) for leaf senescence was applied. Plants were grown in a standard photoperiod with individually darkened leaves. Every day, several darkened leaves were illuminated for 3 h and collected with the control, i.e. untreated ones. *PHOT1* mRNA dark levels in senescent leaves diminished 3-fold during the whole period of senescence, but, an approximately 3-fold drop in transcription after irradiation was preserved on all days except day 4 (Fig. 2A). On the other hand, *PHOT2* mRNA dark levels were generally constant in time, but the upregulation weakened from about 12-fold on day 1 to about 2-fold on day 5 (Fig. 2B).

Multiple photoreceptors are involved in controlling the abundance of phototropin transcripts in mature Arabidopsis leaves

To elucidate the nature of light signal, wild-type plants and photoreceptor-deficient mutants were irradiated with blue or red light. In wild-type *Col-0* plants, both blue and red light downregulated the *PHOT1* transcript abundance, but the effect of blue light was stronger, showing a 2-fold decrease (Fig. 3A). A similar downregulation of *PHOT1* mRNA was observed in *Ler* plants (data not shown). Two mutants used in the study – *phyB* and *phyAphyB* – had much lower *PHOT1* dark levels. In particular, the dark level in the *phyAphyB* mutant was only 25% of that measured in its wild-type counterpart. This mutant also showed lower levels of *PHOT1* mRNA under all conditions tested and the accumulation of its transcript was not diminished by light. A lack of regulation of *PHOT1* mRNA abundance by blue and red light was also observed in the *phyB* single mutant, whereas in *phyA* the expression of *PHOT1* was reduced to about 30% under red light, with almost no effect under blue light. The opposite effect of blue light on the mRNA level of *PHOT1* was detected in the *cry1* mutant. Consequently, the *cry1cry2* mutant also demonstrated no regulation of *PHOT1* transcript abundance by blue light. In all of the cryptochrome-deficient mutants – *cry1*, *cry2*, and *cry1cry2* – the expression of *PHOT1* was downregulated by red light more strongly than in the wild-type *Col-0*. Apart from a slightly bigger influence of red light on the *PHOT1* transcript level, the *phot2* mutant showed a comparable regulation of *PHOT1* transcription to wild-type *Col-0* plants.

The *PHOT2* mRNA levels in wild-type *Col-0* plants were regulated to a comparable level by both blue and red light (Fig. 3B). The *PHOT2* expression increased over 3-fold
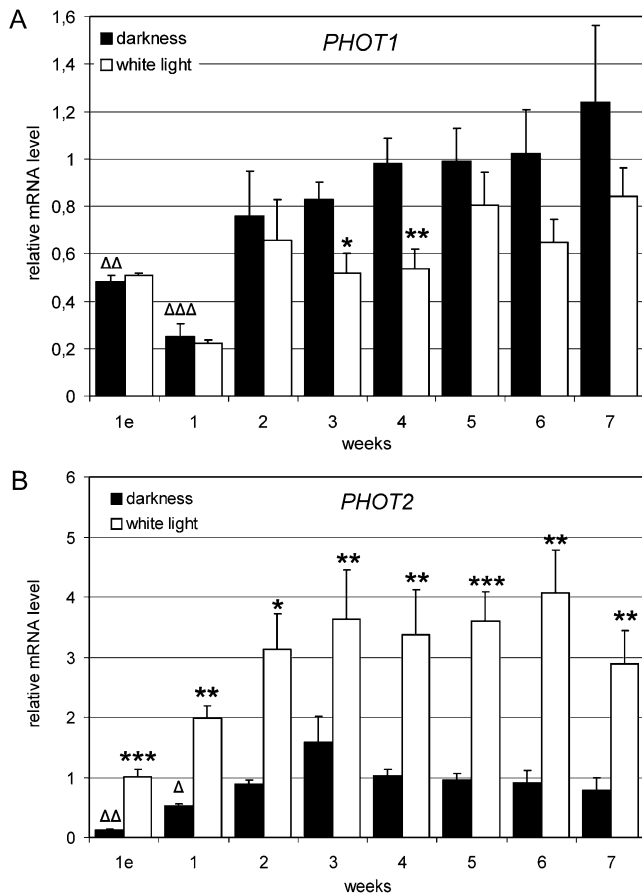

Fig. 1. Changes in the expression of *PHOT1* (A) and *PHOT2* (B) at the mRNA level dependent on the age of *Arabidopsis thaliana*. Every week, whole, dark-adapted plants were illuminated with 100 μmol m^{-2} s^{-1} in the growth chamber for 3 h (light). Controls for seedlings (weeks 1–3) were unexposed plants and for older plants (weeks 4–7) were individual leaves darkened by wrapping in black paper. The relative mRNA levels in darkness (black bars) and after 3 h irradiation (white bars). 1e, 1-week-old etiolated seedlings. Each bar corresponds to an average of at least five measurements from leaves of different plants. Error bars indicate the standard error. Asterisks indicate the statistical significance of the difference between dark- and light-treated samples: *, $P = 0.01$–0.05; **, $P = 0.001$–0.01; ***, $P < 0.001$. Triangles indicate the statistical significance of the difference between dark-adapted samples at a given time and in week 4: Δ, $P = 0.01$–0.05; ΔΔ, $P = 0.001$–0.01; ΔΔΔ, $P < 0.001$.
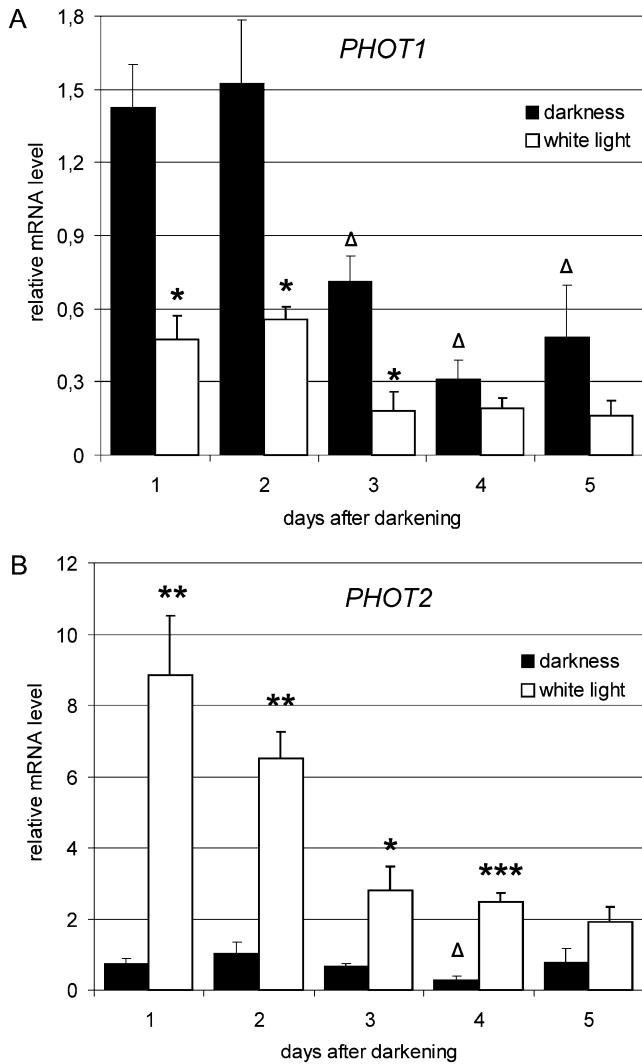
Fig. 2. The expression of *PHOT1* (A) and *PHOT2* (B) at the mRNA level in senescing leaves. Senescence was induced in plants growing in standard photoperiod by wrapping individual leaves in black paper for the indicated number of days (1–5). The relative mRNA levels in darkened leaves (black bars) and after 3 h irradiation with white light of 120 µmol m^{-2} s^{-1} (white bars). Each bar corresponds to an average of at least three measurements from leaves of different plants. Error bars indicate the standard error. Asterisks indicate the statistical significance of the difference between dark- and light-treated samples: *, $P = 0.01–0.05$; **, $P = 0.001–0.01$; ***, $P < 0.001$. Triangles indicate the statistical significance of the difference between dark-adapted samples at a given time and on day 1: Δ, $P = 0.01–0.05$.

Fig. 3. Light-regulated expression of *PHOT1* (A) and *PHOT2* (B) transcripts in *Arabidopsis thaliana* photoreceptor mutants. Each photoreceptor-deficient mutant is indicated under the corresponding bars. The relative mRNA levels in leaves of dark-adapted plants (black bars) and after 3 h irradiation with blue (white bars) or red (grey bars) light of 40 µmol m^{-2} s^{-1}. Each bar represents three technical replicates from the measurement of mRNA isolated from a pool of leaves of at least ten different plants. Error bars indicate the standard error.

after both light treatments. A similar effect was observed for the *Ler* ecotype (data not shown). It is worth noting that the dark levels of *PHOT2* mRNA were diminished by about 2-fold in the *cry1*, *cry1cry2*, *phyB*, and *phyAphyB* mutants. In all phytochrome mutants tested, blue light was observed to have a stronger influence on *PHOT2* mRNA levels than the red light. In the *phyA* mutant, the effect of red light was negligible. A strong, 10-fold blue light upregulation of *PHOT2* transcript occurred in the *phyB* and *phyAphyB* mutants. It should be emphasized that in the *cry1cry2* double mutant the
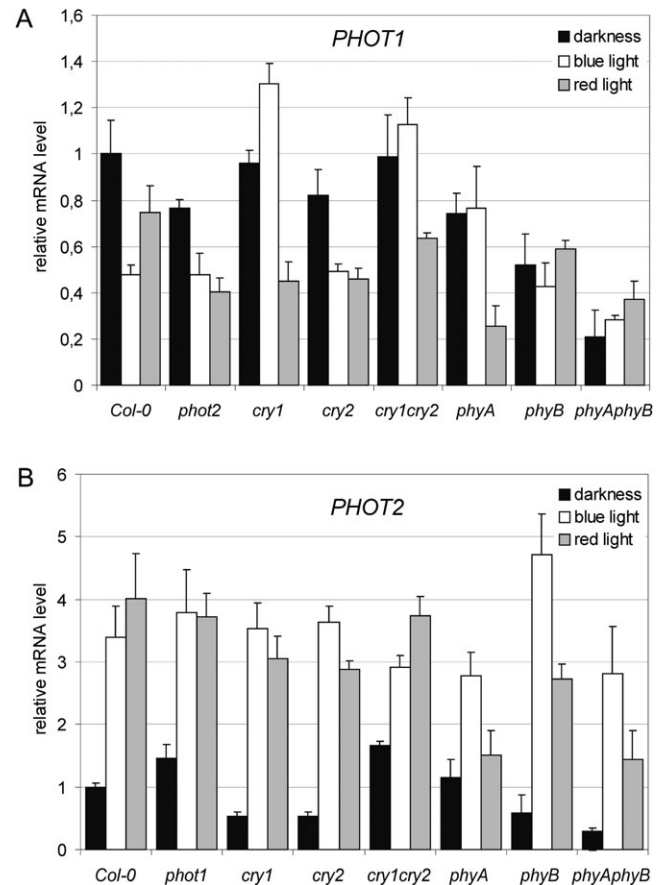
light regulation of *PHOT2* expression was weaker than in the wild-type *Col-0* plants due to the high dark level. On the other hand, the light regulation was stronger in the *cry1* and *cry2* cryptochrome single mutants because of the low dark levels. The modulation of *PHOT2* expression by light was comparable in the *phot1* mutant and in the wild-type *Col-0*.

DCMU affects mRNA levels of PHOT2 but not PHOT1

In order to exclude the influence of photosynthesis on phototropin mRNA levels, DCMU-treated leaves were investigated. DCMU inhibited the quantum efficiency of photosystem II to 40–50% of the initial value (data not shown), which is considered to be sufficient for altering gene expression (Pfannschmidt *et al.*, 2009). This treatment had no effect on *PHOT1* expression. The same, 2-fold downregulation was observed in the control and DCMU-sprayed plants (Fig. 4A). In contrast, DCMU influenced the *PHOT2* transcript levels in darkness and after light exposure. The dark level of *PHOT2* mRNA was about 2-fold lower after DCMU
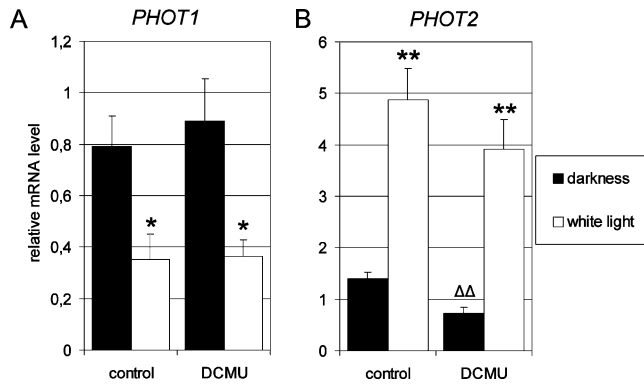
Fig. 4. The influence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) on the expression of *PHOT1* (A) and *PHOT2* (B) at the mRNA level in *Arabidopsis thaliana*. The relative mRNA levels in darkened leaves (black bars) and after 3 h irradiation with white light of 120 µmol m^{-2} s^{-1} (white bars). DCMU, leaves incubated with 200 µM DCMU in 0.4% DMSO; control, plants treated only with 0.4% DMSO. Each bar corresponds to an average of at least five measurements from leaves of different plants. Error bars indicate the standard error. Asterisks indicate the statistical significance of the difference between dark- and light-treated samples: *, $P = 0.01$–0.05; **, $P = 0.001$–0.01. Triangles indicate the statistical significance of the difference between control and DCMU-treated dark-adapted samples: ΔΔ, $P = 0.001$–0.01.

treatment than in control plants. The light level of the *PHOT2* transcript was lower than in control plants, but in relation to the dark level, the light upregulation in DCMU-treated plants was even stronger (Fig. 4B).

Discussion

Analysis of phototropin mRNA levels upon different light treatments shows that the general pattern of light influence on their expression remains unchanged during *Arabidopsis* growth. The only exception is the lack of *PHOT1* transcript regulation in light-grown and etiolated seedlings. These data contrast with the results obtained for 2-day-old *Arabidopsis* seedlings irradiated with 10 µmol m^{-2} s^{-1} of blue light by Kang *et al.* (2008) who reported a 6-fold decrease. It should be noted that the experimental system in the present study was different: 7-day-old seedlings were treated with white light of 100 µmol m^{-2} s^{-1}. In line with the current results, the data from microarrays shows that *PHOT1* mRNA is very weakly downregulated by blue light of 10 µmol m^{-2} s^{-1} in 4-day-old etiolated seedlings (AtGenExpress Light Series from www.arabidopsis.org; Winter *et al.*, 2007). Additionally, the current study found that *EF1α*, used by Kang *et al.* (2008) as an internal control gene, is upregulated by light (Fig. 5), as opposed to the reference genes (*SAND* and *PDF2*) used in this study. To conclude, the effect of phototropin1 downregulation, readily visible at the protein level in 3-day-old seedlings (Sakamoto and Briggs, 2002), should be attributed to regulation primarily at the protein and not the mRNA level. The change in the abundance of
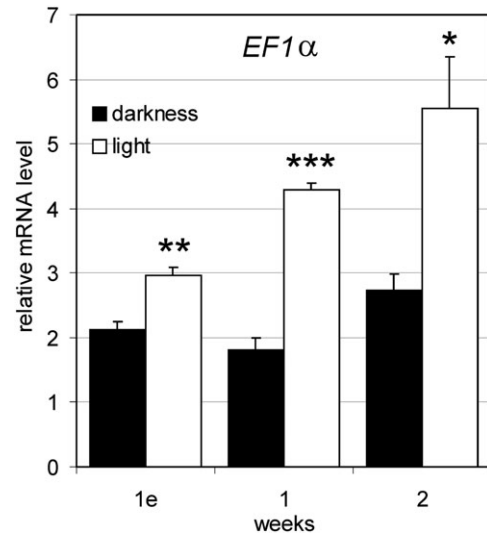

Fig. 5. The mRNA levels of *EF1α* in 1- and 2-week-old seedlings: untreated, dark-adapted ones (black bars) and 3 h irradiated with white light of 100 µmol m^{-2} s^{-1} (white bars). Each bar corresponds to an average of at least five measurements from pools of different plants. The relative mRNA level of *EF1α* was calculated using *SAND* and *PDF* genes as reference. Error bars indicate the standard error. Asterisks indicate the statistical significance of the difference between dark- and light-treated samples: *, $P = 0.01$–0.05; **, $P = 0.001$–0.01; ***, $P < 0.001$.

PHOT1 mRNA upon light exposure seems to be of secondary importance. Recent findings support this idea. In 3-day-old seedlings, phototropin1 is polyubiqiutinated under strong blue light and directed to degradation by the 26S proteasome. This event is considered to be a way of desensitization of the signalling pathway mediated by phot1 (Roberts *et al.*, 2011).

The characteristic transcription pattern of phototropins, i.e. *PHOT1* downregulation and *PHOT2* upregulation by light, is conserved in leaves at different stages of development, even though significant differences exist at the transcription levels. An 'expression burst' of phototropin1 is observed in week 2 after germination and then it remains at an enhanced level until the end of the plant's development. The transcription profile of *PHOT2* is similar. As mentioned earlier, this effect is also observable for phototropins of *Oryza sativa*: the *OsPHOT1* and *OsPHOT2* transcripts are elevated in mature leaves as compared to seedlings (Jain *et al.*, 2007) and for pea phototropin P*sPHOT1a* (Elliot *et al.*, 2004). The change in the phototropin expression levels coincides with the maturation of the leaves. This probably reflects a stronger demand for these photoreceptors and is associated with the acquisition of new biological functions during a plant's development: regulation of stomatal movement, leaf expansion, and chloroplast relocations.

The interesting developmental effect of light regulation in etiolated and light-grown seedlings is that, as reported for *OsPHOT1* and *OsPHOT2* transcripts (Jain *et al.*, 2007), there are higher levels of *PHOT1* during skotomorphogenic growth in contrast to higher levels of *PHOT2* during

photomorphogenic growth. The pea homologue of *PHOT1*, P*sPHOT1b*, also shows a very high expression in etiolated 7-day-old seedlings. These results may suggest that high *PHOT1* transcript abundance is physiologically relevant. Phototropin1 plays a dominant role in low-fluence light responses (starting from 0.01 μmol m^{-2} s^{-1}) by controlling phototropism (Sakai *et al.*, 2001) and rapid inhibition of hypocotyl growth (Folta and Spalding, 2001), so it is an important component for photomorphogenic development. It is possible that dark-grown seedlings accumulate more phototropin1 than the light-grown ones, because they have to sense even very low fluence-rate to direct their growth towards light and to de-etiolate as rapidly as possible.

The performed senescence studies show that the need for both phototropins diminishes during this process. However, the conservation of the light regulation of both transcripts is a striking feature that may be a general mechanism by which the plant adapts to maximize benefits from changing light conditions.

The second part of the study focuses on finding the photoreceptors involved in light control of phototropin mRNA expression. The obtained results show that multiple photoreceptors are involved in controlling the abundance of phototropin transcripts (Fig. 6). In the leaves of 5-week-old plants, *PHOT1* expression is diminished in response to blue and red light. Transcription of *PHOT1* appears to be regulated by cryptochrome1, since the *cry1* mutant does not show the reduction in *PHOT1* mRNA level upon blue light exposure. Impaired blue light regulation occurs also in the *cry1 cry2* double mutant. This result is consistent with the earlier study on *Arabidopsis* seedlings (Kang *et al.*, 2008). PhytochromeB is probably the second photoreceptor active in *PHOT1* mRNA downregulation, because the *phyB* and *phyAphyB* mutants are sensitive neither to red nor to blue

Fig. 6. A model of blue and red light regulation of phototropin transcript abundance in *Arabidopsis* leaves: *PHOT1* (A) and *PHOT2* (B). Down arrows, downregulation of *PHOT* mRNA; up arrows, upregulation of *PHOT* mRNA: broader arrows indicate stronger regulation. BL, blue light; RL, red light; dashed lines, less evident modulation.

light. This photoreceptor seems to be responsible also for *PHOT1* transcript abundance, as both *phyB* and *phyAphyB* have low *PHOT1* mRNA levels in darkness. Both phytochromes, A and B, play a role in the blue light modulation of *PHOT1* transcript accumulation, as none of the phytochrome mutants examined – *phyA*, *phyB*, or *phyAphyB* – are sensitive to this treatment. Regarding the data on blue light regulation of *PHOT1* expression, an interaction of phytochromes A and B with cryptochrome1 should be postulated (Fig. 6A). This kind of interaction is well known from physiological studies of seedlings (for review, see Casal, 2000). Moreover, it was also postulated at the transcriptional level, in activation of the light-responsive element of a small subunit of Rubisco from *Nicotiana* (Martínez-Hernández *et al.*, 2002).

Blue light enhances *PHOT2* expression in leaves of wild-type *Arabidopsis*; a comparable effect is also observed in red light. Two groups of photoreceptors seem to contribute to the observable effects, cryptochromes and phytochromes, as a lesser blue light regulation of *PHOT2* mRNA occurs in the *cry1cry2* double mutant and no effect of red light on the *PHOT2* expression is observed in the *phyA* mutant. However, phyB or other phytochromes not examined in this study may also influence this process (Fig. 6B). Like the *PHOT1* transcript abundance, *PHOT2* mRNA levels in darkness appear to be controlled mainly by phytochromeB.

It should be emphasized that phototropins do not cross-regulate their mRNA levels in leaves subjected to light treatment. This finding is consistent with results obtained at the protein level (Kimura and Kagawa, 2009).

The last part of this study deals with the role of plastid retrograde signalling in the control of phototropin transcription, by analysing the effects of DCMU – a typical inhibitor of photosynthesis (Pfannschmidt *et al.*, 2009). While no influence on *PHOT1* transcript abundance is observed, the *PHOT2* mRNA levels seem to be affected both in darkness and after light exposure. This effect is stronger in the absence of light than after illumination, suggesting that it might be unrelated to photosynthesis. In yeast, DCMU can influence the respiratory chain, as an inhibitor of the cytochrome bc$_1$ complex (Convent *et al.*, 1978). However, this is not likely in plant systems. A study on isolated potato tuber cytochrome bc$_1$ showed only 25% inhibition at 500 μM DCMU, indicating that in plants this complex is significantly more resistant (Berry *et al.*, 1991). Concerning the regulation of *PHOT2*, one may speculate that the signal from chloroplasts, reflecting their redox state, might activate the pathway of phototropin2 synthesis to fulfil an enhanced need for this photoreceptor to sense light and cope with its excess more efficiently. As phototropin2 is the major photoreceptor involved in controlling the avoidance response of chloroplasts (Jarillo *et al.*, 2001; Kagawa *et al.*, 2001), which plays a photoprotective role (Sztatelman *et al.*, 2010), this hypothesis remains tempting, but needs further research for validation.

The obtained data indicate that the regulation pattern of phototropins is important for their functioning, as it remains conserved almost in all conditions tested. However,
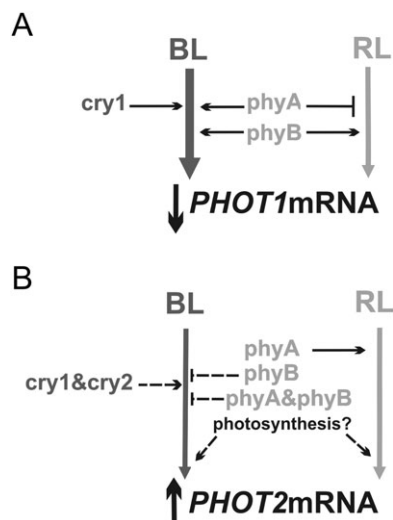
the physiological impact of this phenomenon still remains to be elucidated. Some studies that focus on the protein level postulate that the expression of phototropins determines the sensitivity to light of phototropin-mediated responses. The velocity of chloroplast avoidance movement and phototropism were associated with the level of phototropin2 in transgenic lines (Kimura and Kagawa, 2009). A similar effect was reported for phototropin1: a dose-dependent restoration of phot1 controlled processes (Doi *et al.*, 2004). The above studies were performed with phototropins expressed under the control of 35S promoter in *phot*-deficient mutants. This puts in question the physiological relevance of the observed effects. Moreover, another study postulates that the sensitivity of phototropism is determined by the activity of phototropin1 rather than by its expression level (Aihara *et al.*, 2008). Further studies are needed to integrate the information about light regulation of phototropin mRNA profiles with the protein content and photoreceptor functioning.

Acknowledgements

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