

RESEARCH PAPER

# AUXIN-BINDING-PROTEIN1 (ABP1) in phytochrome-B-controlled responses

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## Abstract

The auxin receptor ABP1 directly regulates plasma membrane activities including the number of PIN-formed (PIN) proteins and auxin efflux transport. Red light (R) mediated by phytochromes regulates the steady-state level of ABP1 and auxin-inducible growth capacity in etiolated tissues but, until now, there has been no genetic proof that ABP1 and phytochrome regulation of elongation share a common mechanism for organ elongation. In far red (FR)-enriched light, hypocotyl lengths were larger in the *abp1-5* and *abp1/ABP1* mutants, but not in *tir1-1*, a null mutant of the TRANSPORT-INHIBITOR-RESPONSE1 auxin receptor. The polar auxin transport inhibitor naphthylphthalamic acid (NPA) decreased elongation in the low R:FR light-enriched white light (WL) condition more strongly than in the high red:FR light-enriched condition WL suggesting that auxin transport is an important condition for FR-induced elongation. The addition of NPA to hypocotyls grown in R- and FR-enriched light inhibited hypocotyl gravitropism to a greater extent in both *abp1* mutants and in *phyB-9* and *phyA-211* than the wild-type hypocotyl, arguing for decreased phytochrome action in conjunction with auxin transport in *abp1* mutants. Transcription of FR-enriched light-induced genes, including several genes regulated by auxin and shade, was reduced 3–5-fold in *abp1-5* compared with Col and was very low in *abp1/ABP1*. In the *phyB-9* mutant the expression of these reporter genes was 5–15-fold lower than in Col. In *tir1-1* and the *phyA-211* mutants shade-induced gene expression was greatly attenuated. Thus, ABP1 directly or indirectly participates in auxin and light signalling.

**Key words:** AUXIN-BINDING PROTEIN1 (ABP1), early auxin-regulated genes, elongation, gravitropism, phototropism, phytochrome, shade avoidance.

## Introduction

Auxin initiates responses by at least two different receptors, AUXIN BINDING PROTEIN1 (ABP1) and TRANSPORT INHIBITOR RESPONSE1 (TIR1) (Scherer, 2011). TIR1 mediates auxin effects on gene expression (Mockaitis and Estelle, 2008), while ABP1 mediates auxin effects at the plasma membrane (Napier *et al.*, 2002; Robert *et al.*, 2010; Xu *et al.*, 2010). ABP1 is essential for development and many rapid cellular changes (Jones *et al.*, 1998; Chen

*et al.*, 2001a, b). ABP1-mediated rapid responses such as membrane hyperpolarization, channel regulation, proton extrusion, phospholipase A activation (Scherer and André, 1989; Labusch *et al.*, 2013), phospholipase D activation, transient increase in cytosolic calcium and elongation are too rapid to be reconciled with TIR1 as the only auxin receptor, assuming that the sole function of TIR1 is mediating changes in gene transcription through its degradation

Abbreviations: ABP1, AUXIN-BINDING PROTEIN1; B, blue; FR, far red; NPA, naphthylphthalamic acid; PIN, PIN-FORMED protein; R, red; TIR1, TRANSPORT-INHIBITOR-RESPONSE1; WL, white light; wt, wild type.

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of transcriptional regulators (Badescu and Napier, 2006; Scherer, 2011).

ABP1 is a small glycoprotein localized in the ER lumen with 1–3% secreted to the extracytosolic side of the plasma membrane where it binds auxin (Tian *et al.*, 1995; Napier *et al.*, 2002). The *ABP1* expression pattern is strongly overlapping with that of the artificial auxin-activated *DR5* promoter coupled to the *uidA* gene (Klode *et al.*, 2011) suggesting a causal relationship between ABP1 action and auxin concentrations, consistent with the observation that auxin regulates *ABP1* transcription (Hou *et al.*, 2006; Effendi *et al.*, 2011). In order to transmit signalling to cytosolic proteins, a transmembrane protein, ‘docking protein’ or binding protein for ABP1, was postulated (Klämbt, 1990). A critical feature of hormone receptors is that the activated pool size limits the amplitude and/or rate of signal transduction at physiological concentrations of the cognate hormone (Kenakin, 2004). Consistent with the ABP1 number being rate-limiting for auxin responses, null *abp1* mutants are embryo lethal (Chen *et al.*, 2001b) and the heterozygous *abp1/ABP1* mutant displays auxin-signalling defects (Effendi *et al.*, 2011). It was speculated that proper stoichiometry of ABP1 and the hypothetical binding protein is rate-limiting for signal output and any disturbance of this stoichiometry causes a mutant auxin phenotype. This gene dosage effect or haploinsufficiency (Veitia *et al.*, 2008) is common for receptors in humans (Fisher and Scambler, 1994). A dosage effect for ABP1 function was also demonstrated using conditional deletion by expressing a recombinant antibody fragment directed against ABP1, a line designated *abp1-SS12* (Braun *et al.*, 2008). Additional observations that active ABP1 is rate-limiting are: (i) the level of ABP1 and auxin-induced growth capacity is correlated in tobacco leaves (Chen *et al.*, 2001b), (ii) genetic ablation of ABP1 blocks embryogenesis at an early phase when auxin induces the elongation of the top tier of cells (Chen *et al.*, 2001b), and (iii) reduction of ABP1 reduces auxin-induced expansion without an effect on auxin-induced cell division (Jones *et al.*, 1998).

Most, if not all, phenotypes associated with *ABP1* mutations are linked to a malfunction of polar auxin transport conducted or regulated by PIN proteins (Robert *et al.*, 2010; Xu *et al.*, 2010; Effendi *et al.*, 2011; Effendi and Scherer, 2011). PIN1 proteins are located on the plasma membranes along the tips of epidermal cell lobes and are linked to the expansion of lobes in an auxin signalling pathway that uses ABP1 as a receptor and small G proteins as intermediates (Xu *et al.*, 2010). At these positions, the level of auxin is critical for the proper development of pavement cells (Xu *et al.*, 2010). Robert *et al.* (2010) showed that ABP1 is the receptor for the auxin-inhibition of endocytosis of PIN proteins. As a consequence, the efflux transport by these PIN proteins is enhanced (Paciorek *et al.*, 2005). Another example of a possible link between ABP1 and polar auxin transport is the correlation of ABP1, auxin concentration, and H<sup>+</sup>-ATPase localization in embryo development (Chen *et al.*, 2010). It was shown, in particular, that the heterozygous T-DNA insertion mutant *abp1/ABP1* has defects in (i) root and hypocotyl gravitropism, (ii) basipetal auxin transport in the root,

(iii) apical dominance, and (iv) regulation of early auxin-activated genes (Effendi *et al.*, 2011). In our model, these functions were linked to the regulation of auxin transport which, in turn, regulates the auxin concentrations perceived by the extracytosolic ABP1 receptor and the nuclear receptor TIR1 (Effendi *et al.*, 2011; Effendi and Scherer, 2011; Scherer *et al.*, 2012).

Red (R) and blue (B) light decreases auxin transport, steady-state ABP1 level, and auxin-binding capacity (Shinkle and Jones, 1988; Jones *et al.*, 1991; Shinkle *et al.*, 1992, 1998; Barker-Bridges *et al.*, 1998; Liu *et al.*, 2011). R decreased the steady-state level of ABP1 and auxin transport over a time-course consistent with the kinetics of R-induced decrease in hypocotyl elongation. Other light-regulated physiological responses involve auxin transport and require ABP1. Increased hypocotyl elongation in FR-enriched light, and expression of rapidly R- or FR-induced genes were all different in *abp1-5* and *abp1/ABP1* compared with wild types (wt). Further, impeding elongation and gravitropism in hypocotyls by the auxin transport inhibitor naphthylphenylphthalamic acid (NPA) revealed the impact of auxin transport on these phytochrome-controlled responses as proposed (Robson and Smith, 1996; Jensen *et al.*, 1998; Keuskamp *et al.*, 2010; Kozuka *et al.*, 2010). Thus, ABP1 plays a direct or indirect role in the shade avoidance response in *Arabidopsis* and it is speculated that ABP1 regulates auxin transport as part of the mechanism.

## Materials and methods

### Plant material and growth conditions

Heterozygous kanamycin-resistant *abp1/ABP1* mutant seeds (Chen *et al.*, 2001b) are in a Ws background and the genotypes verified as before (Chen *et al.*, 2001b; Effendi *et al.*, 2011). *abp1-5* contains a mutation of a conserved histidine to a tyrosine (H94Y) (Robert *et al.*, 2010) in the auxin-binding pocket of ABP1 (Woo *et al.*, 2002). *phyA-211* and *phyB* are in the Col-0 background and were obtained from M Zeidler, and *tir1-1* and *tir1-9* were obtained from M Quint.

For the gravitropism and phototropism experiments, seeds were stratified for 4 d, treated for 4 h with WL and grown for 3 d vertically on 0.5× MS agar plates in the dark at 22.5 °C. For testing gravitropism, plants were turned 90° for 24 h and then scanned. Lateral blue light at 10 μmol·m<sup>-2</sup>·s<sup>-1</sup> (CLF, Plant Climatics) was applied and scanned after 8 h (CanonScan 8800F; resolution 600 dots per inch). For testing shade avoidance, seeds were stratified for 4 d, treated with WL for 4 h, and then kept in the dark for 24 h. Thereafter, WL (14.5 μmol m<sup>-2</sup>·s<sup>-1</sup>) was applied for 3 d, followed by WL supplemented with R and FR either with a high R:FR ratio (2.11) or a low R:FR ratio (0.098) in an LED box at 22.5 °C (CLF, Plant Climatics) for another 3 d at 22.5 °C or on NPA-containing agar or 1 h for subsequent RNA isolation. Hypocotyl lengths or angles were measured using AxioVision LE Ver.4.6 software (Zeiss-Germany). For flowering time experiments, plants were grown in a growth chamber at 22.5 °C in 8/16 h (L/D). Each experiment was done at least twice. Where necessary, heterozygous *abp1/ABP1* plants were identified by genotyping as before (Chen *et al.*, 2001b; Effendi *et al.*, 2011).

### Nucleic acid analysis

For transcription measurements, seedlings were grown in 0.5× MS agar-medium for 14 d in long (12/12 h) days. For the auxin treatment,

the medium was removed and replaced by fresh medium containing 10  $\mu$ M 1-NAA. Seedlings were blotted on filter paper and frozen in liquid nitrogen for further use. For quantitative RT-PCR, 4  $\mu$ g of total RNA was prepared with the NucleoSpin<sup>®</sup> RNA Plant kit according to the manufacturer's instructions (Macherey and Nagel) and transcribed to first strand cDNA with RevertAid<sup>™</sup> H Minus First Strand cDNA Synthesis kit (Fermentas). Primers and methods were as described previously (Effendi *et al.*, 2011; Effendi and Scherer, 2011; the primers are listed in Supplementary Table S1 at *JXB* online). For each data point, two to five biological repeats and three technical replicates for each determination were done in the subsequent PCR reaction. Relative expression was calculated according to the  $\Delta\Delta C_t$  method using the equation: relative expression =  $2^{-[\Delta C_t(\text{sample}) - \Delta C_t(\text{control})]}$ , with  $\Delta C_t = C_{t(\text{sample gene})} - C_{t(\text{reference gene})}$ , where  $C_t$  refers to the threshold cycle determined for each gene in the early exponential amplification phase (Livak and Schmittgen, 2001). The control treatment at  $t=0$  min was set as 1-fold expression level. For statistical analysis the REST 2008 software (Pfaffl *et al.*, 2002) was used.

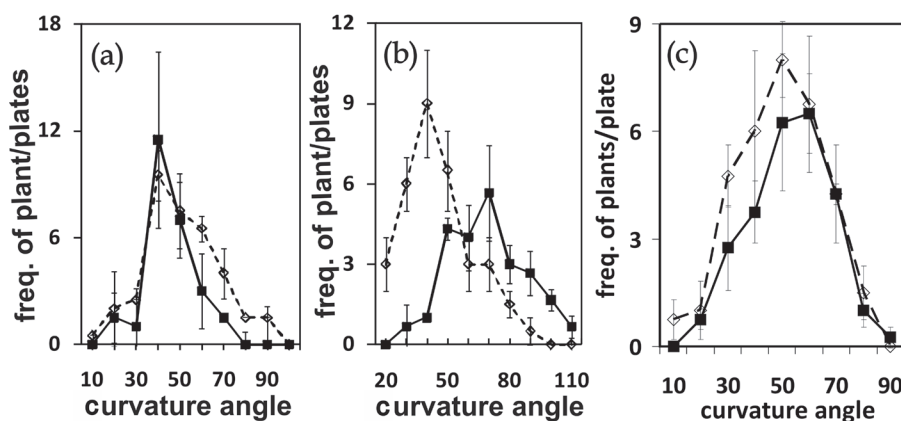
## Results

The mutant *abp1-5* containing a histidine 94 $\rightarrow$ tyrosine point mutation has near-normal morphology (see Supplementary Fig. S1 and Fig. S2 at *JXB* online; data not shown). As shown in Supplementary Fig. S2 at *JXB* online, both flowering time and the number of rosette leaves at the beginning of flowering were nearly identical in *abp1-5* and in the wild type in short days in contrast to *abp1/ABP1* (Effendi *et al.*, 2011). Although, the gravitropic response of hypocotyls and the phototropic response to laterally applied blue light of hypocotyls of *abp1-5*, grown in the dark, was statistically indistinguishable from the wild type (Fig. 1a, c), the gravitropic response in roots was less than the wild type (Fig. 1b). *abp1/ABP1* seedlings had an agravitropic and an aphototropic phenotype (Effendi *et al.*, 2011). To a lesser extent as in *abp1/ABP1* (Effendi *et al.*, 2011), delayed expression of several auxin-inducible genes was found in *abp1-5* (see Supplementary Fig. S3 at *JXB* online) confirming that ABP1 affects auxin function(s).

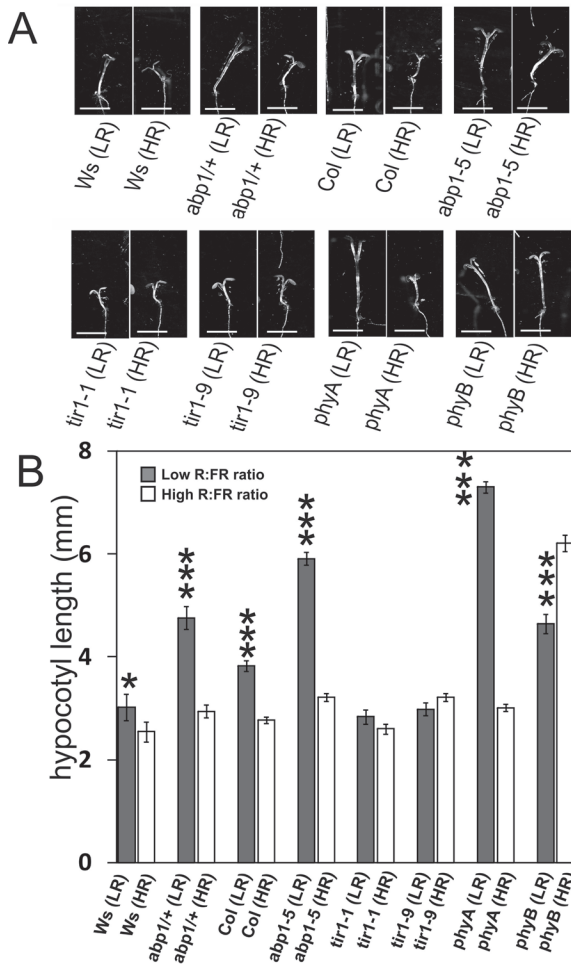
Accelerated hypocotyl elongation is characteristic of the shade avoidance response in plants and depends on auxin transport (Jensen *et al.*, 1998). In both *abp1-5* and in *abp1/ABP1* mutant seedlings, the response to FR-enriched light was tested and compared with the response in *tir1* mutants. Plants were grown first in WL for 3 d and either continued with augmented R light to create a high red:far red (R:FR) ratio (non-shade) or at a low R:FR ratio (shade) for another 3 d (spectra in Supplementary Fig. S4 at *JXB* online). Hypocotyl elongation in both *abp1* mutants were significantly taller in FR-enriched light than in the wild type. The respective wild types showed a much smaller elongation response to low R:FR (Fig. 2). In high R:FR ratio conditions, the *abp1* mutants were like the wild type.

TIR1 regulates gene transcription by auxin-stimulated ubiquitination of AUX/IAA proteins which are negative co-transcription factors (Mockaitis and Estelle, 2008). Therefore, two *tir1* alleles, *tir1-1* and *tir1-9*, were also tested for their elongation response to shade conditions (Fig. 2). In contrast to *abp1* mutants, hypocotyl lengths of *tir1-1* and *tir1-9* in both low and high R:FR conditions were not significantly different and they exhibited no shade response.

R and FR abrogate hypocotyl gravitropism and the inhibition of hypocotyl gravitropism depends on active  $P_t$  of either phyA or phyB (Liscum and Hangarter, 1993; Robson and Smith, 1996) and NPA, originally described as a gravitropic inhibitor (Geissler *et al.*, 1985), has become a diagnostic tool for auxin transport. As shown in Fig. 3, *abp1* mutants and phytochrome mutants lose their gravitropic orientation in both low and high R:FR ( $P < 0.01$ ) with the exception of *phyB* in low ratio R:FR light (versus Col) and the effect of NPA was similar on *abp1* and *phy* mutants. The effect of NPA on elongation induced in low R:FR light was also tested (Jensen *et al.*, 1998; Steindler *et al.*, 1999; Kozuka *et al.*, 2010) and it was compared with the effect of NPA on elongation in high ratio R:FR light in the *abp1* mutants and *phyA* and *phyB* mutants (see Supplementary Fig. S5 at *JXB* online). Greater



**Fig. 1.** Gravitropic and phototropic responses in 3-d-old dark-grown Col-0 (black squares) and *abp1-5* (diamonds) seedlings. (a) Gravitropic bending angles of hypocotyls after 24 h tilting by 90° (mean Col: 44.8°;  $n=57$ ; mean *abp1-5*: 46.7°;  $n=42$ ;  $P < 0.54$ ; difference not significant). (b) Gravitropic bending angles of roots after 24 h tilting by 90° (mean Col: 65.3°;  $n=71$ ; mean *abp1-5*: 41.1°;  $n=65$ ;  $P < 0.001$ ). (c) Phototropic bending angles of hypocotyls after 8 h lateral blue light (10  $\mu$ mol  $m^{-2} s^{-1}$ ) (mean Col: 48.9°;  $n=135$ ; mean *abp1-5*: 45.7°;  $n=102$ ;  $P < 0.114$ ; difference not significant). For each panel, 3–4 agar plates containing about 30 seedlings were evaluated. Data points represent means of each angle size group and SE.



**Fig. 2.** Shade-avoidance responses in *abp1-5* and *abp1/ABP1* compared with *Col*, *phyA-211*, and *phyB-9*. Shade avoidance was tested by growing seedlings for 3 d in WL and for 3 more days in WL or white plus added low R:FR ratios (LR, simulated shade) or high ratios of R:FR (HR, non-shade). Seedlings from seeds from an *abp1/ABP1* plant were verified by PCR-genotyping as either *Ws* wild type or *abp1/ABP1* mutant (Effendi et al., 2011). For comparison, *phyA-211* and *phyB-9* mutants were added to the tests. (A) Representative seedlings of every line used grown in low or high ratio of FR:R. Bar=5 mm. (B) The hypocotyl lengths of seedlings grown in low (dark bars) or high ratio (white bars) of R:FR. Hypocotyl lengths of seedlings were evaluated. LR and HR treatments were statistically different except for the *tir1* alleles. Significance levels in (B): \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; ( $n=55-90$ ; SE).

NPA inhibition was simply associated with taller hypocotyls, a sensitivity difference in mutants or wild types to NPA concentration was small if any.

To test the hypothesis that ABP1 is involved in the shade-avoidance response, the expression of shade-induced marker genes was quantified after 1 h to narrow down the time at which the reorganization of transcription by the interaction of *abp1-5* and phytochromes occurs (Fig. 4a–g). Several FR-light-regulated genes in the shade response (*ATHB2*, *PIL1*, *PIF5*, *HFRI1*) and of auxin- and light-regulated genes (*IAA19*, *IAA29*, *PIN3*) were quantified (Devlin et al., 2003;

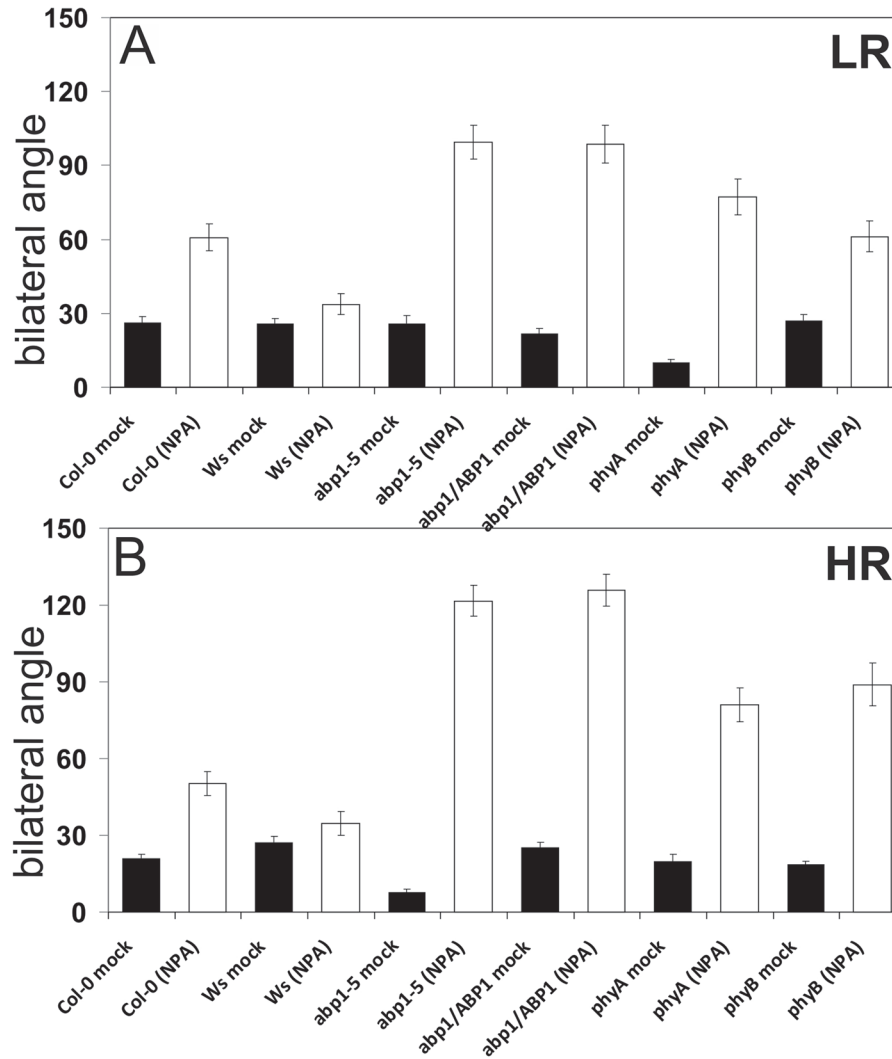
Salter et al., 2003; Sessa et al., 2005; Roig-Villanova et al., 2006; Tepperman et al., 2006; Hornitschek et al., 2009; Keuskamp et al., 2010; Kunihiko et al., 2011). After 3 d in WL, seedlings were treated for 1 h with WL either enriched with FR (low ratio R:FR or shade) where phyB is inactive or with R (high ratio R:FR) where phyB is active (Fig. 4). As a control, seedlings that were treated with WL only were set as 1-fold expression. After only 1 h light in shade conditions, expressions of the tested shade marker genes were, in general, higher, consistent with Tepperman et al. (2006). In *abp1-5*, induction by shade was about 4–8-fold lower than in *Col* and in *abp1/ABP1* induction was low compared with *Ws*. In *phyB*, the induction of expression by 1 h low R:FR was 8–15-fold lower than in *Col*. In *tir1-1*, the induction of *ATHB2* was low and the induction of *IAA29* was higher than in all other genotypes. In *phyA*, *ATBH2* induction was high and that of *IAA29* was modest and only these two genes were noticeably induced. *ATBH2* and *IAA29* were also induced by low R:FR light in *tir1-1* so that the overall pattern in *tir1-1* was somewhat similar to that in *phyA* but dissimilar to *Col*.

The expression of the tested genes in high R:FR conditions was generally low or absent in *Col* or *phyA* (Fig. 4h, j) compared with *abp1-5* and *abp1/ABP1* or the *phyB* mutants (Fig. 4i, k, m) and low in *Ws* and in *tir1* (Fig. 4l, n). In *abp1-5*, *abp1/ABP1* or *phyB* several genes at least were induced. Again, this can be interpreted as a decrease in the phyB control of repressing genes in *abp1* mutants similar to that in *phyB* (Jiao et al., 2007). Interestingly, in high R:FR conditions *TAA1* expression, an auxin biosynthesis gene (Tao et al., 2008), was very high in *phyB* (80 $\times$ ) compared with *Col*, *phyA*, or *tir1* but still high in *abp1-5* (15 $\times$ ) although it was modest in *Ws* or *abp1/ABP1*. Together, the data suggest that *TAA1* expression is repressed by phyB and repression is absent in shade or in *phyB* seedlings in the high R:FR condition. Regardless of the photoreceptor mechanism, regulation of light-regulated genes was clearly disturbed in *abp1-5*, *abp1/ABP1*, and *tir1-1*.

## Discussion

Shade avoidance is a complex trait involving inputs from light and hormones, especially auxin. The shade-avoidance response is induced in plants by sensing a low R:FR ratio in the WL background. The shade-avoidance response is primarily sensed by phyB (Reed et al., 1993) induced by a low R:FR ratio, although phyD and phyE participate to some degree in sensing (Aukerman et al., 1997; Devlin et al., 1998; Devlin et al., 1999). Low signalling activity by CRY1 in low B light also contributes to the shade-avoidance response (Ballaré, 2009; Kunihiko et al., 2010). Our physiological results and our results on auxin-induced gene expression (Fig. 1; see Supplementary Fig. S3 at JXB online) show that *abp1-5* is an auxin signalling mutant just as is *abp1/ABP1* (Effendi et al., 2011) and both have the capacity to modulate red light responses.

Based on published observations (Shinkle and Jones, 1988; Jones et al., 1991; Shinkle et al., 1992, 1998; Barker-Bridges



**Fig. 3.** Diagnostic effects of 5  $\mu$ M NPA on hypocotyl gravitropic orientation in (A) low and (B) high ratio R:FR light in *abp1-5* and *abp1/ABP1* and phytochrome mutants and wild types. Data are from 24 to 54 seedlings per assay (SE). The genotype of *abp1/ABP1* plants was verified by PCR. In LR Col and *phyB* seedlings in the presence of NPA were not statistically significant different but *phyA* seedlings were different from Col ( $P < 0.05$ ). In HR, all mutants in the presence of NPA were significantly different from the wild types ( $P < 0.01$  or lower).

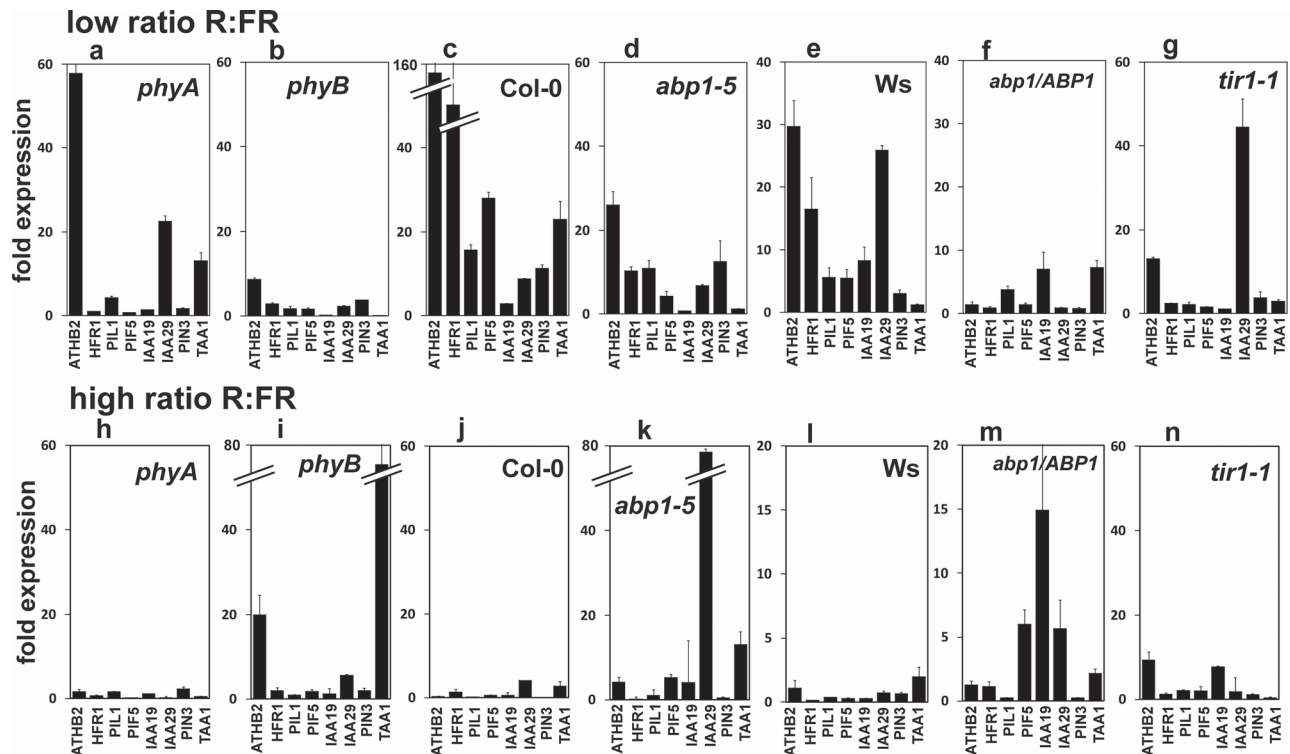
*et al.*, 1998; Robert *et al.*, 2010; Xu *et al.*, 2010; Effendi *et al.*, 2011) and the data presented here, it is illustrated in Fig. 5 that one important nexus linking auxin and R signalling is ABP1. Since ABP1 is not a cytoplasmic protein, any direct interaction with phyB is unexpected. However, ABP1-mediated auxin signalling through the aforementioned ABP1 docking protein and downstream factors may regulate phyB-dependent signalling. Inhibition of the growth repressing regulatory activity of phyB is the predominant mechanism.

#### *ABP1 and predominantly phyB link auxin and red light physiology*

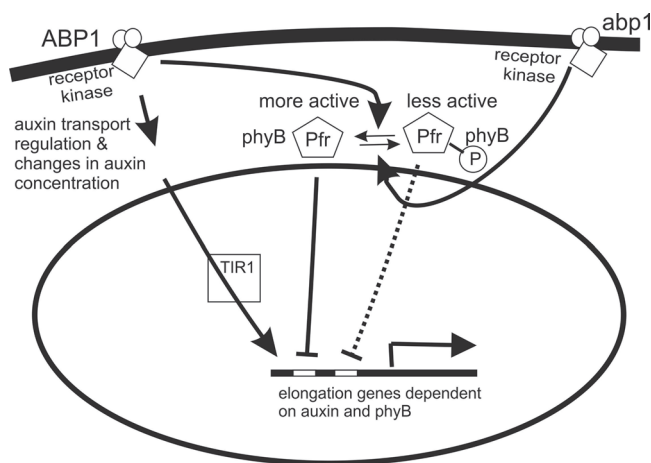
Increased elongation in low ratio R:FR light is a hallmark of the response of plants to physiological shade and low signalling output in this light by phyB is recognized to be the main reason (Reed *et al.*, 1993; Stamm and Kumar, 2010). The *tir1* alleles did not respond to low ratio R:FR conditions (Fig. 2).

With respect to hypocotyl elongation *abp1-5* and *abp1/ABP1* resemble weak *phyB* mutants (Fig. 2) in that they hyperelongate in low ratio R:FR conditions compared with the shade responses of their wild types. However, the insensitivity to R as seen in the response of *phyB* to high ratio R:FR was not observed in them.

NPA applied under red light revealed that *abp1* mutants phenocopy phytochrome mutants in their loss of gravitropic orientation (Fig. 3). Hypocotyl gravitropism requires asymmetrical auxin transport (Friml *et al.*, 2002; Nagashima *et al.*, 2008a, b). Gravitropism is inhibited by R and FR and thus phyB and phyA are the relevant photoreceptors identified in continuous R or FR light (Liscum and Hangarter, 1993; Robson and Smith, 1996). Inhibition of hypocotyl gravitropism by phytochromes in our experiments was evidenced by a comparison of *phyA* and *phyB* seedlings with the *abp1* mutants with and without NPA (Fig. 3). We did not use R or FR alone but with added WL all genotypes grew without



**Fig. 4.** Comparison of regulation of genes by low ratio R:FR (a–f, shade) and high ratio R:FR (g–l, non-shade) in Col, *phyA*, *phyB*, *abp1-5*, and *tir1-1*. Seedlings were tested by growing for 3 d in WL and for 1 h in WL or white plus added low R:FR ratios or high ratios of R:FR. Expression was normalized to  $t=0$  in WL only and set as 1-fold for either genotype. Error bars were calculated according to Pfaffl et al. (2002) and are significant when not overlapping ( $P < 0.05$  or lower). Genotype of *abp1/ABP1* plants was verified by PCR prior to RNA isolation.



**Fig. 5.** A working model of the functional interaction of ABP1 and phytochrome B. ABP1 interacts with a postulated transmembrane docking protein (Klämbt, 1990; Scherer et al., 2012) capable of transmitting the auxin signal across the membrane. This could be a receptor kinase or a calcium channel (or other) so that a post-translational modification of phyB as described by Medzihradsky et al. (2013) seems a possibility for functional interaction. According to the authors, comparison of *phyB-9* plants expressing phospho-mimic yellow fluorescent fusion protein phyB<sup>Ser86Asp</sup>-YFP or nonphosphorylatable phyB<sup>Ser86Ala</sup>-YFP demonstrated that phosphorylation of Ser-86 negatively regulates all physiological

NPA almost completely upright and any red light effect was small. Increased randomization of hypocotyls in *phyA*, *phyB*, and *abp1* mutant lines in the presence of NPA indicated that *abp1* mutants, in general, behaved as weak phenocopies of phytochrome-deficient seedlings (Fig. 3). Whether *phyA* or *phyB* or signalling from both phytochromes was affected in the *abp1* mutants cannot be decided but, clearly, auxin transport was disturbed in this loss of gravitropic orientation and NPA acted as an enhancer. Although PIN proteins are known to regulate gravitropism and expression analysis of the DR5:GUS auxin reporter gene in *pin3* seedlings suggested that they are impaired in the normal lateral transport during tropism (Friml et al., 2002), it is clear that NPA also

*phyB* responses tested by them including the response to shade. Light-independent relaxation of the phosphomimicking phyB<sup>Ser86Asp</sup> Pfr into phyB<sup>Ser86Asp</sup> Pr (dark reversion) is strongly enhanced both *in vivo* and *in vitro*. Faster dark reversion attenuates red light-induced nuclear import and interaction with the negative regulator PHYTOCHROME INTERACTING FACTOR3 compared with the wild-type version phyB<sup>Ser86</sup>-GFP (Medzihradsky et al., 2013). It is suggested that ABP1 can influence this phosphorylation–dephosphorylation equilibrium towards the more active form. This more active form can still be inactivated by FR so that wt ABP1 plants show a small elongation to shade whereas the *abp1* mutants show a hyper-response.

impairs the asymmetric distribution of auxin in hypocotyl tropism in an ABCB19-dependent manner (Nagashima *et al.*, 2008b). The proteins actually binding NPA are the ABCB transporters (Bailly *et al.*, 2011). ABCB19 transporter mutants are agravitropic (Noh *et al.* 2001; Blakeslee *et al.* 2007; Nagashima *et al.* 2008b) and in red light their hypocotyl orientation randomizes (Nagashima *et al.*, 2008a). PIN proteins act co-operatively with ABCB proteins (Blakeslee *et al.*, 2007; Bailly *et al.*, 2011) so that PINs in tropisms may also act in a co-operative manner with the ABCB auxin transporters. In monochromatic R light ABCB19 and ABCB1 protein expression decreases (Nagashima *et al.*, 2008a, b). Adding NPA in our experiments probably further reduced their activity leading to strong randomization. In conclusion, auxin transport components and red light sensors interact in the inhibition of hypocotyl gravitropism and this interaction is disturbed in *abp1* mutants pointing out an ABP1 and phytochrome interaction.

#### Light-induced gene expression in *abp1* mutants

Expression patterns of known shade-induced genes in low ratio R:FR (shade) and high ratio R:FR light support our hypothesis that ABP1 and phyB are linked in red light signalling. Since kinetics is so important in the argument, it is noteworthy that this difference could be detected as early as one hour after the start of shade. At about this time point, shade-induced elongation starts to become apparent (Cole *et al.*, 2011; Li *et al.*, 2012).

Our hypothesis of functional interaction of ABP1 and phyB is further supported by data on the expression of shade-induced genes. Compared with Col, the induction of the shade marker genes (*ATHB2*, *HFR1*, *PIF1*, *PIF5*) is much lower in *abp1-5* and very low in *phyB* and also low in *abp1/ABP1* compared with Ws (Fig. 4). *IAA19* and *PIN3*, both of which are induced by auxin and (to a low extent) in shade in wt and *abp1-5*, were not induced in *abp1/ABP1*. Lack of expression of shade-induced genes in high ratio R:FR demonstrate that, in Col and *phyA* (being wt with respect to phyB), expression was mostly repressed but in *phyB*, *abp1-5*, and *abp1/ABP1* some genes escaped light repression (Fig. 4h–n).

The *tir1-1* mutant showed altogether a different pattern of regulation of light-induced genes than either Col, *abp1-5*, or *abp1/ABP1* with none of the genes tested here being induced by shade except *IAA29* (Fig. 4). While *IAA29* was highly induced in *tir1* compared with Col or *abp1* mutants, *IAA29* is not ubiquitinated by TIR1 (Dreher *et al.*, 2006; Maraschin *et al.*, 2009) so it might escape control by TIR1 in *tir1*. Defects in the co-regulation of genes induced by light and by auxin, as noted before (Devlin *et al.*, 2003; Kunihiro *et al.*, 2011; Stamm and Kumar, 2010), is a possibility that could explain this lack of shade response in the *tir1* mutant and the hyperelongation of *abp1* mutants. The lack of shade-induced elongation in *tir1* probably indicates the necessity of activation of further auxin-regulated genes than those tested here for sustained elongation and other members of the TIR/AFB family may act redundantly with TIR1 in this.

#### Auxin biosynthesis, auxin transport, and shade response

One important auxin input into the shade-avoidance response is an increase in auxin signal strength by the shade-dependent induction of *TAA1* transcription, an auxin biosynthesis gene (Tao *et al.*, 2008). Our findings confirm this for low R:FR light in the wild type but, in *abp1-5*, *TAA1* is only induced a little in *abp1/ABP1* (Fig. 4) and not at all in *phyB* and *tir1*. This does not correlate with the hypocotyl lengths of these genotypes in shade light. Further, how the early timing of the transcriptional response of *TAA1* translates into an increase of IAA is still unclear (Quint *et al.*, 2009; Mashiguchi *et al.*, 2011; Mana and Nemoto, 2012). So it is also unclear how exactly auxin concentration makes its input into the shade responses (Stamm and Kumar, 2010; Nozue *et al.*, 2011).

A potential shared element of auxin and phyB signalling in the shade-avoidance syndrome may be PIN3 (Keuskamp *et al.*, 2010; Kozuka *et al.*, 2010). As discussed above PIN3 and ABCB transporters probably co-operatively participate in their responses to auxin and light (Blakeslee *et al.*, 2007; Nagashima *et al.*, 2008a; Bailly *et al.*, 2011). Rapid regulation of ABCB is not so well investigated as that of PIN proteins. The regulation of PIN proteins may occur as protein subcellular re-distribution most rapidly (Kleine-Vehn and Friml, 2008) and/or at the transcriptional level (Vieten *et al.*, 2005; Effendi and Scherer, 2011). Auxin modulates auxin transport within a few minutes independently of transcriptional regulation (Paciorek *et al.*, 2005; Petrasek *et al.*, 2006; Robert *et al.*, 2010). Specifically, the regulation of *PIN3* and perhaps other *PIN* genes could be part of a common set of intermediates between ABP1 and phyB. Consistent with this notion, the expression of *ABCB19* is repressed by R although modes of interaction in shade of ABCB19 and PIN3 are unknown (Nagashima *et al.*, 2008a, b). ABP1 regulates polar auxin transport at the organ level (Effendi *et al.*, 2011) and by the regulation of *PIN3* expression (Effendi and Scherer, 2011). Exactly how phyB (or/and phyA) enters into the ABP1 pathway remains mostly unclear. However, recently Medzihradzky *et al.* (2013) showed that phosphorylation of phyB inhibits light-induced signalling. The transmembrane protein postulated by Klämbt (1990) to interact with ABP1 could have the necessary enzymatic activity, for example, a protein kinase or a calcium channel stimulating calcium-dependent phosphorylation, to transmit a phosphorylation as the signal to activate phyB. Our working model (Fig. 5) presented here provides a launching point to dissect the recently-speculated cytosolic phytochrome signalling pathway (Rösler *et al.*, 2010).

#### Supplementary data

Supplementary data can be found at *JXB* online.

Supplementary Fig. S1. Auxin sensitivity of *abp1-5*.

Supplementary Fig. S2. Flowering date in Col-0 and *abp1-5* plants grown in short days (16/8 h L/D).

Supplementary Fig. S3. Rapid regulation of early auxin genes by 10  $\mu$ M 1-NAA in Col-0 wild type and *abp1-5* mutant seedlings.

**Supplementary Fig. S4.** Spectra used in the shade-avoidance experiments.

**Supplementary Fig. S5.** Effect of NPA on elongation in (A) low ratio (R:FR) supplemented WL or (B) in high ratio (R:FR) supplemented WL light.

**Supplementary Table S1.** List of primers.

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## References

- Aukerman MJ, Hirschfeld M, Wester L, Weaver M, Clack T, Amasino RM, Sharrock RA.** 1997. A deletion in the PHYD gene of the Arabidopsis Wassilewskija ecotype defines a role for phytochrome D in red/far-red light sensing. *The Plant Cell* **9**, 1317–1326.
- Badescu GO, Napier RM.** 2006. Receptors for auxin: will it all end in TIRs?. *Trends in Plant Sciences* **11**, 217–223.
- Ballaré CL.** 2009. Illuminated behaviour: phytochrome as a key regulator of light foraging and plant anti-herbivore defence. *Plant, Cell and Environment* **32**, 713–725.
- Bailly A, Yang H, Martinoia E, Geisler M, Murphy AS.** 2011. Plant lessons: exploring ABCB functionality through structural modeling. *Frontiers in Plant Science* **2**, 108.
- Barker-Bridges M, Ribnicky DM, Cohen JD, Jones AM.** 1998. Red-light regulated growth. II. Changes in the abundance of indoleacetic acid in the maize mesocotyl. *Planta* **204**, 207–211.
- Blakeslee JJ, Bandyopadhyay A, Lee OR, et al.** 2007. Interactions among PIN-FORMED and P-glycoprotein auxin transporters in Arabidopsis. *The Plant Cell* **19**, 131–147.
- Braun N, Wyrzykowska J, Muller P, David K, Couch D, Perrot-Rechenmann C, Fleming AJ.** 2008. Conditional repression of AUXIN BINDING PROTEIN1 reveals that it coordinates cell division and cell expansion during postembryonic development in Arabidopsis and Tobacco. *The Plant Cell* **20**, 2746–2762.
- Chen D, Ren Y, Deng Y, Zhao J.** 2010. Auxin polar transport is essential for the development of zygote and embryo in *Nicotiana tabacum* L. and correlated with ABP1 and PM H<sup>+</sup>-ATPase activities. *Journal of Experimental Botany* **61**, 1853–1867.
- Chen J-G, Shimomura S, Sitbon F, Sandberg G, Jones AM.** 2001a. Role of auxin-binding protein 1 in leaf cell growth. *The Plant Journal* **28**, 607–617.
- Chen JG, Ullah H, Young JC, Sussman MR, Jones AM.** 2001b. ABP1 is required for organized cell elongation and division in Arabidopsis embryogenesis. *Genes and Development* **15**, 902–911.
- Cole B, Kay SA, Chory J.** 2011. Automated analysis of hypocotyl growth dynamics during shade avoidance in Arabidopsis. *The Plant Journal* **65**, 991–1000.
- Devlin PF, Patel SR, Whitelam GC.** 1998. Phytochrome E influences internode elongation and flowering time in Arabidopsis. *The Plant Cell* **10**, 1479–1487.
- Devlin PF, Robson PR, Patel SR, Goosey L, Sharrock RA, Whitelam GC.** 1999. Phytochrome D acts in the shade-avoidance syndrome in Arabidopsis by controlling elongation growth and flowering time. *Plant Physiology* **119**, 909–915.
- Devlin PF, Yanovsky MJ, Kay SA.** 2003. A genomic analysis of the shade avoidance response in Arabidopsis. *Plant Physiology* **133**, 1617–1629.
- Dreher KA, Brown J, Saw RE, Callis J.** 2006. The Arabidopsis Aux/IAA protein family has diversified in degradation and auxin responsiveness. *The Plant Cell* **18**, 699–714.
- Effendi Y, Rietz S, Fischer U, Scherer GFE.** 2011. The heterozygous *abp1/ABP1* insertional mutant has defects in functions requiring polar auxin transport and in regulation of early auxin-regulated genes. *The Plant Journal* **65**, 282–294.
- Effendi Y, Scherer GFE.** 2011. AUXIN BINDING-PROTEIN1 (ABP1), a receptor to regulate auxin transport and early auxin genes in an interlocking system with PIN proteins and the receptor TIR1. *Plant Signaling and Behavior* **6**, 1101–1103.
- Fisher E, Scambler P.** 1994. Human haploinsufficiency: one for sorrow, two for joy. *Nature Genetics* **7**, 5–7.
- Friml J, Wisniewska J, Benkova E, Mendgen K, Palme K.** 2002. Lateral relocation of auxin efflux regulator PIN3 mediates tropism in Arabidopsis. *Nature* **415**, 806–809.
- Geissler AE, Pilet PE, Katekar GF.** 1985. Growth and gravireaction of maize roots treated with a phytotropin. *Journal of Plant Physiology* **119**, 25–34.
- Hornitschek P, Lorrain S, Zoete V, Michielin O, Fankhauser C.** 2009. Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. *EMBO Journal* **28**, 3893–3902.
- Hou HW, Zhou YT, Mwange KN, Li WF, He XQ, Cui KM.** 2006. ABP1 expression regulated by IAA and ABA is associated with the cambium periodicity in *Eucommia ulmoides* Oliv. *Journal of Experimental Botany* **57**, 3857–3867.
- Jensen PJ, Hangarter RP, Mark E.** 1998. Auxin transport is required for hypocotyl elongation in light-grown but not dark-grown Arabidopsis. *Plant Physiology* **116**, 455–462.
- Jiao Y, Lau OS, Deng XW.** 2007. Light-regulated transcriptional networks in higher plants. *Nature Reviews Genetics* **8**, 217–230.
- Jones AM, Cochran DS, Lamerson PL, Cohen J, Evans M.** 1991. Red-light induced changes in auxin, an auxin-binding protein, and auxin transport in maize mesocotyl. *Plant Physiology* **97**, 352–358.
- Jones AM, Im KH, Savka MA, Wu MJ, DeWitt NG, Shillito R, Binns AN.** 1998. Auxin-dependent cell expansion mediated by overexpressed auxin-binding protein 1. *Science* **282**, 1114–1117.
- Kenakin T.** 2004. Principles: receptor theory in pharmacology. *Trends in Pharmacological Sciences* **25**, 186–192.



- Keuskamp DH, Pollmann S, Voeselek LACJ, Peeters AJM, Pierik R.** 2010. Auxin transport through PIN-FORMED 3 (PIN3) controls shade avoidance and fitness during competition. *Proceedings of the National Academy of Sciences, USA* **107**, 22740–22744.
- Klämbt D.** 1990. A view about the function of auxin-binding proteins at plasma membranes. *Plant Molecular Biology* **14**, 1045–1050.
- Kleine-Vehn J, HFrml JH.** 2008. Polar targeting and endocytic recycling in auxin-dependent plant development. *Annual Review of Cell and Developmental Biology* **24**, 447–473.
- Klode M, Dahlke RI, Sauter M, Steffens B.** 2011. Expression and subcellular localization of *Arabidopsis thaliana* Auxin-Binding Protein 1 (ABP1). *Journal of Plant Growth Regulation* **30**, 416–424.
- Kozuka T, Kobayashi J, Horiguchi G, Demura T, Sakakibara H, Tsukaya H, Nagatani A.** 2010. Involvement of auxin and brassinosteroid in the regulation of petiole elongation under the shade. *Plant Physiology* **153**, 1608–1618.
- Kunihiro A, Yamashino T, Mizuno T.** 2010. PHYTOCHROME-INTERACTING FACTORS PIF4 and PIF5 are implicated in the regulation of hypocotyl elongation in response to blue light in *Arabidopsis thaliana*. *Bioscience, Biotechnology and Biochemistry* **74**, 2538–2541.
- Kunihiro A, Yamashino T, Nakamichi N, Niwa Y, Nakanishi H, Mizuno T.** 2011. PHYTOCHROME-INTERACTING FACTOR 4 and 5 (PIF4 and PIF5) activate the homeobox *ATHB2* and auxin inducible *IAA29* genes in the coincidence mechanism underlying photoperiodic control of plant growth of *Arabidopsis thaliana*. *Plant and Cell Physiology* **52**, 1315–1329.
- Labusch C, Shishova M, Effendi Y, Li M, Wang X, Scherer GF.** 2013. Patterns and timing in expression of early auxin-induced genes imply involvement of phospholipases A (pPLAs) in the regulation of auxin responses. *Molecular Plant* March 21. [Epub ahead of print]
- Li L, Ljung K, Breton G, et al.** 2012. Linking photoreceptor excitation to changes in plant architecture. *Genes and Development* **26**, 785–790.
- Liscum E, Hangarter RP.** 1993. Genetic evidence that the red absorbing form of phytochrome B modulates gravitropism in *Arabidopsis thaliana*. *Plant Physiology* **103**, 15–19.
- Liu X, Cohen JD, Gardner G.** 2011. Low-fluence red light increases the transport and biosynthesis of auxin. *Plant Physiology* **157**, 891–904.
- Livak KJ, Schmittgen TD.** 2001. Analysis of relative gene expression data using real time quantitative PCR and the  $2^{-\Delta\Delta Ct}$  method. *Methods* **25**, 402–408.
- Mano Y, Nemoto K.** 2012. The pathway of auxin biosynthesis in plants. *Journal of Experimental Botany* **63**, 2853–2872.
- Maraschin FS, Memelink J, Offringa R.** 2009. Auxin-induced, SCF(TIR1)-mediated poly-ubiquitination marks AUX/IAA proteins for degradation. *The Plant Journal* **59**, 100–109.
- Mashiguchi K, Tanaka K, Sakai T, et al.** 2011. The main auxin biosynthesis pathway in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **108**, 18512–18517.
- Medzihradzky M, Bindics J, Ádám É, et al.** 2013. Phosphorylation of phytochrome B inhibits light-induced signaling via accelerated dark reversion in *Arabidopsis*. *The Plant Cell* **25**, 535–544.
- Mockaitis K, Estelle M.** 2008. Auxin receptors and plant development: a new signaling paradigm. *Annual Review of Cellular Developmental Biology* **24**, 55–80.
- Nagashima A, Suzuki G, Uehara Y, et al.** 2008a. Phytochromes and cryptochromes regulate the differential growth of *Arabidopsis* hypocotyls in both a PGP19-dependent and a PGP19-independent manner. *The Plant Journal* **53**, 516–529.
- Nagashima A, Uehara Y, Sakai T.** 2008b. The ABC subfamily B auxin transporter AtABCB19 is involved in the inhibitory effects of *N*-1-naphthylphthalamic acid on the phototropic and gravitropic responses of *Arabidopsis* hypocotyls. *Plant and Cell Physiology* **49**, 1250–1255.
- Napier RM, David KM, Perrot-Rechenmann C.** 2002. A short history of auxin-binding proteins. *Plant Molecular Biology* **49**, 339–348.
- Noh B, Murphy AS, Spalding EP.** 2001. Multidrug resistance-like genes of *Arabidopsis* required for auxin transport and auxin-mediated development. *The Plant Cell* **13**, 2441–2454.
- Nozue K, Harmer SL, Maloof JN.** 2011. Genomic analysis of circadian clock-, light-, and growth-correlated genes reveals PHYTOCHROME-INTERACTING FACTOR5 as a modulator of auxin signaling in *Arabidopsis*. *Plant Physiology* **156**, 357–372.
- Paciorek T, Zazimalová E, Rudthardt N, et al.** 2005. Auxin inhibits endocytosis and promotes its own efflux from cells. *Nature* **435**, 1251–1256.
- Petrášek J, Mravec J, Bouchard R, et al.** 2006. PIN proteins perform a rate-limiting function in cellular auxin efflux. *Science* **12**, 914–918.
- Pfaffl MW, Horgan GW, Dempfle L.** 2002. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Research* **30**, e36.
- Quint M, Barkawi LS, Fan K-T, Cohen JD, Gray WM.** 2009. *Arabidopsis IAR4* modulates auxin response by regulating auxin homeostasis. *Plant Physiology* **150**, 748–758.
- Reed JW, Nagpal P, Poole DS, Furuya M, Chory J.** 1993. Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. *The Plant Cell* **5**, 147–157.
- Robert S, Kleine-Vehn J, Barbez E, Sauer M, et al.** 2010. ABP1 mediates auxin inhibition of clathrin-dependent endocytosis in *Arabidopsis*. *Cell* **143**, 111–121.
- Robson PRH, Smith H.** 1996. Genetic and transgenic evidence that phytochrome A and B act to modulate the gravitropic orientation of *Arabidopsis thaliana* hypocotyls. *Plant Physiology* **110**, 211–216.
- Roig-Villanova I, Bou J, Sorin C, Devlin PF, Martínez-García JF.** 2006. Identification of primary target genes of phytochrome signaling. Early transcriptional control during shade avoidance responses in *Arabidopsis*. *Plant Physiology* **141**, 85–96.
- Rösler J, Jaedicke K, Zeidler M.** 2010. Cytoplasmic phytochrome action. *Plant and Cell Physiology* **51**, 1248–1254.
- Salter MG, Franklin KA, Whitelam GC.** 2003. Gating of the rapid shade-avoidance response by the circadian clock in plants. *Nature* **426**, 680–683.

- Scherer GFE.** 2011. AUXIN-BINDING-PROTEIN1, the second auxin receptor: what is the significance of a two-receptor concept in plant signal transduction? *Journal of Experimental Botany* **62**, 3339–3357.
- Scherer GFE, André B.** 1989. A rapid response to a plant hormone: auxin stimulates phospholipase  $A_2$  *in vivo* and *in vitro*. *Biochemical and Biophysical Research Communications* **163**, 111–117.
- Scherer GF, Labusch C, Effendi Y.** 2012. Phospholipases and the network of auxin signal transduction with ABP1 and TIR1 as two receptors: a comprehensive and provocative model. *Frontiers of Plant Science* **3**, 56.
- Sessa G, Carabelli M, Sassi M, Ciolfi A, Possenti M, Mittempergher F, Becker J, Morelli G, Ruberti I.** 2005. A dynamic balance between gene activation and repression regulates the shade avoidance response in Arabidopsis. *Genes and Development* **19**, 2811–2815.
- Shinkle JR, Jones RL.** 1988. Inhibition of stem elongation in *Cucumis* seedlings by blue light requires calcium. *Plant Physiology* **86**, 960–966.
- Shinkle JR, Kadakia R, Jones AM.** 1998. Dim-red-light induced increase in polar auxin transport in cucumber seedlings: I. Development of altered capacity, velocity, and response to inhibitors. *Plant Physiology* **116**, 1505–1513.
- Shinkle JR, Sooudi SK, Jones RL.** 1992. Adaptation to dim-red light leads to a non-gradient pattern of stem elongation in *Cucumis* seedlings. *Plant Physiology* **99**, 808–811.
- Stamm P, Kumar PP.** 2010. The phytohormone signal network regulating elongation growth during shade avoidance. *Journal of Experimental Botany* **61**, 2889–2903.
- Steindler C, Matteucci A, Sessa G, Weimar T, Ohgishi M, Aoyama T, Morelli G, Ruberti I.** 1999. Shade avoidance responses are mediated by the ATHB-2 HD-zip protein, a negative regulator of gene expression. *Development* **126**, 4235–4245.
- Tao Y, Ferrer JL, Ljung K, et al.** 2008. Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell* **133**, 164–176.
- Tepperman JM, Hwang YS, Quail PH.** 2006. phyA dominates in transduction of red-light signals to rapidly responding genes at the initiation of Arabidopsis seedling de-etiolation. *The Plant Journal* **48**, 728–742.
- Tian H, Klämbt D, Jones AM.** 1995. Auxin-binding protein 1 does not bind auxin within the endoplasmic reticulum despite this being the predominant subcellular location for this hormone receptor. *Journal of Biological Chemistry* **270**, 26962–26969.
- Veitia RA, Bottani S, Birchler JA.** 2008. Cellular reactions to gene dosage imbalance: genomic, transcriptomic and proteomic effects. *Trends in Genetics* **24**, 390–397.
- Vieten A, Vanneste S, Wiśniewska J, Benková E, Benjamins R, Beeckman T, Luschnig C, Friml J.** 2005. Functional redundancy of PIN proteins is accompanied by auxin-dependent crossregulation of PIN expression. *Development* **132**, 4521–4531.
- Woo EJ, Marshall J, Baulry J, Chen JG, Venis M, Napier RM, Pickersgill RW.** 2002. Crystal structure of auxin-binding protein 1 in complex with auxin. *EMBO Journal* **21**, 2877–2885.
- Xu T, Wen M, Nagawa S, Fu Y, Chen JG, Wu MJ, Perrot-Rechenmann C, Friml J, Jones AM, Yang Z.** 2010. Cell surface- and Rho GTPase-based auxin signaling controls cellular interdigitation in Arabidopsis. *Cell* **143**, 99–110.