Klarifikasi: (lampiran bukti kehadiran, artikel dll)

Poster yang berjudul: “Isolation and Characterization of New Point Mutants of AUXIN BINDING PROTEIN 1“ dipublikasikan dalam bentuk poster presentation pada International conference: Botaniker Tagung 2011 yang diselenggarakan oleh Frei Universitaet Berlin – Jerman, 18-23 September 2011. Kontributor author adalah sebagai penulis utama (First author). Konferensi ini tidak menyediakan adanya proseding sehingga publikasi ini **tergolong tidak dimuat dalam prosiding**. Informasi tentang konferensi (paniti penyelenggara, peserta dan poster/makalah yang dipresentasikan) terinfokan pada book of abstract (dilampirkan). Sebagai bukti keikutsertaan kami dilampirkan dokumen sebagai berikut:

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BOTANIKER TAGUNG 2011
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CERTIFICATE OF ATTENDANCE

This is to certify that

Yunus Effendi

has attended the

Botanikertagung 2011 Berlin

Diversity makes the difference - September 18-23, Berlin

Organizing Comitte

Prof. Dr. Reinhard Kunze

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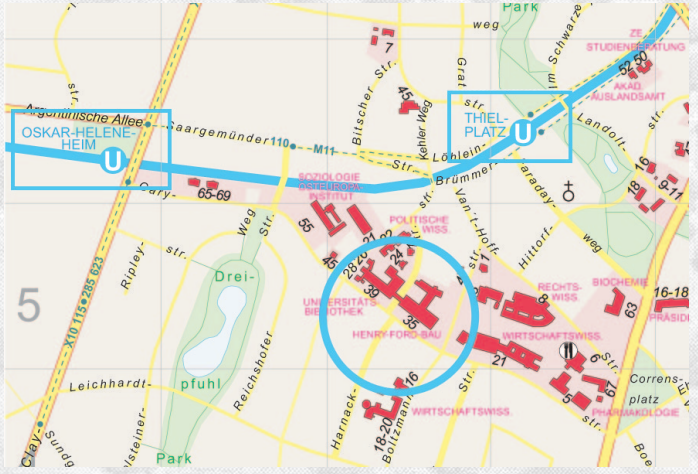
CONGRESS VENUE

The Botanikertagung 2011 will be held in the Henry Ford Building (HFB) on the campus of the Freie Universität Berlin in Berlin-Dahlem. The HFB offers state-of-the-art audio-visual facilities in all lecture halls and can be conveniently reached by subway from downtown Berlin or by bus/subway from Potsdam.

Henry-Ford-Bau (Reinhard Görner)

The conference dinner will be held in the Botanic Garden, Berlin-Dahlem. With its 126 acres, 16 greenhouses and more than 22,000 plant species, the Botanic Garden is internationally renowned for its extensive collections and unique exhibitions.



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The complete program, updates about the conference, a list of hotels and further information are available at the conference website

www.botanikertagung2011.de

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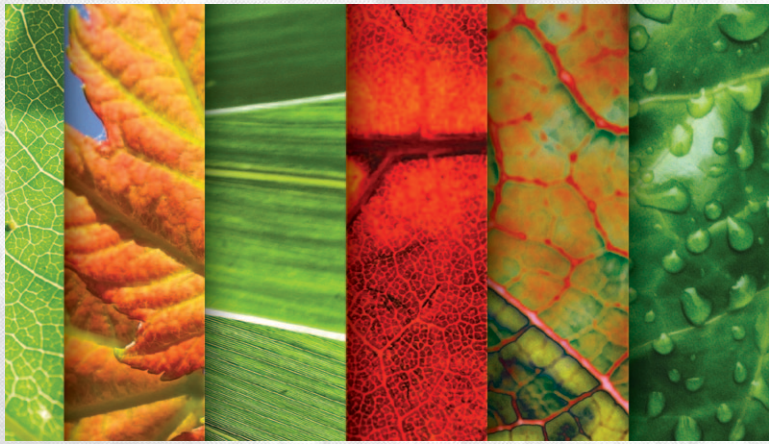


BOTANIKER TAGUNG **2011**

>DIVERSITY MAKES THE DIFFERENCE<

18 - 23 September 2011 - Berlin - Germany

www.botanikertagung2011.de

BOTANIKERTAGUNG 2011

The Botanikertagung is an international Botanical Congress held every two years under the auspices of the Deutsche Botanische Gesellschaft (DBG). The Botanikertagung 2011 is jointly organized by the Freie Universität Berlin and the Humboldt-University Berlin.

SCOPE

Under the title Diversity Makes the Difference the congress offers a multifaceted program that will bring together scientists and students from all fields of plant sciences, including phycology and mycology. A broad spectrum of topics will be covered, ranging from ecology to genome evolution and focusing on innovative and future-oriented concepts and approaches.

PRELIMINARY PROGRAM

Plenary speakers

Ian Baldwin (DE)
Philip Benfey (US)
Jeffry Chen (US)
Joanne Chory (US)
Erwin Grill (DE)
Eva Kondorosi (FR)
Peter Langridge (AU)
Robert Last (US)
Jian-Ren Shen (JP)
Kazuo Shinozaki (JP)
Klaas Jan van Wijk (US)
Dani Zamir (IL)
Daniel Zilberman (US)

Evening Public Lecture

Wilhelm Gruissem (CH)

Symposia

Please visit the conference website for further information regarding the confirmed keynote speakers.

Workshop: Seed Biology

SYMPOSIA TOPICS

- Developmental biology: Organ formation, patterning and differentiation
- Sexual reproduction and flower development
- Growth regulation: Cell cycle, expansion, division
- Organelles: Biogenesis, function, communication
- Plant respiratory control: hypotheses and mechanisms
- Plant mineral nutrition metabolism
- Secondary metabolism
- Membranes: Structure, functions and dynamics
- Plant cellular biology - from protein homeostasis to protein trafficking and targeting
- Photosynthesis: Electron transport chain
- Photo protection, photo inhibition and redox control
- Photosynthesis: Carbon assimilation, C4 and CAM plants
- Cyanobacterial photosynthesis
- Light sensing, light signaling, and circadian rhythm
- Rapid intracellular responses in (receptor-mediated) stress signalling
- Plant hormones: Metabolism, signalling and function
- Abiotic stress: Responses, acclimation, tolerance
- Biotic Stress: Host-pathogen interactions, attack and defence
- Global changes in terrestrial and aquatic ecosystems
- Molecular aspect of algae: From environment to physiology and cell biology
- Biotic stress: Plant defence against herbivores
- Plant-microbe symbiosis and mykorrhiza
- Crop improvement: Agronomic traits and optimized plant usage
- Green biotechnology
- Gene regulatory networks - transcriptional control
- Posttranscriptional control of gene expression: Non-coding RNAs, RNA processing and translation
- Epigenetics
- Heterosis
- New -omics technologies and applications
- Systems biology
- Molecular systematics
- Biodiversity and evolution: Genome stability, plasticity and remodeling
- Environmental context of evolution and speciation
- Evolution of secondary metabolism

SCHEDULE AND DEADLINES

24 January 2011	Online registration and abstract submission opens
31 May 2011	Deadlines for early bird registration and abstracts to be considered for oral presentations
15 July 2011	Deadline for abstract submission
9 September	Deadline for online registration
18 -22 September	Congress
23 September	Excursions

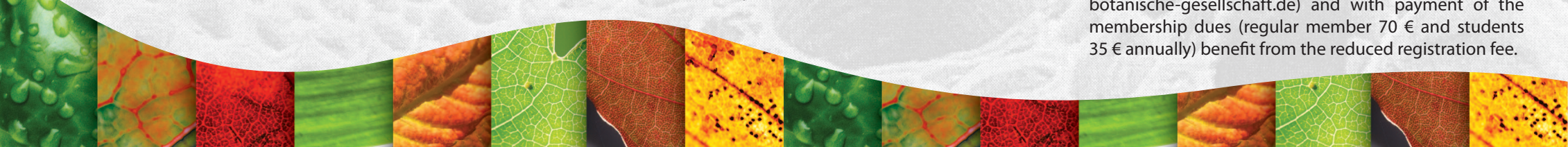
REGISTRATION

Online registration opens on 24 January 2011 at www.botanikertagung2011.de .

	Non-DBG-Members		DBG-Members		
	before 31 May	after 31 May	before 31 May	after 31 May	**day ticket**
Business	380 €	460 €	310 €	390 €	120 €
University/ Research	310 €	370 €	240 €	300 €	90 €
Student	135 €	175 €	100 €	140 €	45 €
Conference Dinner	35 €	35 €	35 €	35 €	

Payment is possible by bank transfer (preferred) or credit card. Students will be asked to show a valid student ID at the registration desk.

Non-members can become new members by applying to the Deutsche Botanische Gesellschaft (www.deutsche-botanische-gesellschaft.de) and with payment of the membership dues (regular member 70 € and students 35 € annually) benefit from the reduced registration fee.



GENERAL INFORMATION

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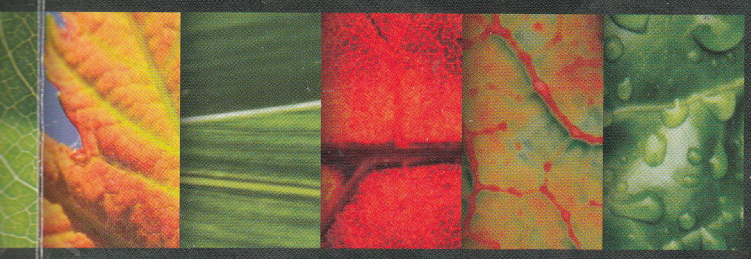

BOTANIKER TAGUNG 2011

CONFERENCE BOOK

POSTER ABSTRACTS | SESSION 27

S27 - POSTER -1

Patatin-related phospholipase A knockout mutants have defects in regulation of early auxin-induced genes

Corinna Labusch (1), Maria Shishova (2), Yunus Effendi (1), Günther F.E. Scherer (1)

(1) Leibniz University of Hannover; (2) University of St. Petersburg

In *Arabidopsis*, a family of ten phospholipase A genes has been identified and are involved in auxin and pathogen signaling (Rietz et al., 2010, Mol. Plant). Plant PLA activity is rapidly induced by different external signals and the PLA reaction products function as second messengers in plant signal transduction (Scherer et al., 2010, TIPS). Here we used the knockout mutants of all ten pPLAs to test the regulation of early auxin genes. Test genes were IAA-genes, SAUR-genes, genes involved in lateral root formation (Péret et al., 2009) and PIN-genes. Many of the lateral root genes and the SAUR genes showed a strong defect in gene expression in the pPLA knockouts after 10 µM auxin application (t=30min). In comparison, the transcription of pPLA genes themselves is not auxin regulated within 30 min. The pPLA knockouts did not show any phenotypes under normal growth conditions or when grown on auxin containing medium. In summary, the pPLA knockouts show a transient mis-regulation of early auxin regulated genes that mostly disappeared after 3 hours. Because the abp1/ABP1 mutant regulated none of early auxin-induced genes at 30 min we hypothesize that ABP1 and PLAs act in the same auxin signaling pathway influencing TIR1 activity in an unknown way (Effendi et al., 2011, Plant J.).

S27 - POSTER -2

The Cytokinin Receptors of *Arabidopsis thaliana* Localize Predominantly to the Endoplasmic Reticulum

Sergey N. Lomin (1), Klaas Wulfetange (2), Alexander Heyl (2), Georgy A. Romanov (1), Thomas Schmülling (2)

(1) Institute of Plant Physiology RAS; (2) Freie Universität Berlin

The plant hormone cytokinin is perceived by membrane-located sensor histidine kinases. *Arabidopsis thaliana* possesses three cytokinin receptors: AHK2, AHK3 and CRE1/AHK4. We investigated the subcellular location of the *Arabidopsis* cytokinin receptors by three different approaches. Cytokinin binding studies with plant membrane fractions separated by two-phase partitioning showed that in the wild type as well as in mutants retaining only single cytokinin receptors the major part of cytokinin-specific binding was associated with endomembranes. Cytokinin binding properties of plant membranes were similar to those found upon heterologous expression of receptors in a bacterial system. The transient expression of receptor-GFP fusion proteins or bimolecular fluorescence complementation analysis in leaf epidermal cells of *Nicotiana benthamiana* showed strong fluorescence of the endoplasmic reticulum (ER) network for each of the receptors. To detect Myc-tagged receptors in membrane fractions by immunoblotting, transgenic lines expressing recombinant genes under their own promoters were generated. Separation of the microsomal fraction by sucrose gradient centrifugation followed by immunoblotting showed a Mg^{2+}-dependent density shift of cytokinin receptors typical of ER membrane proteins.

S27 - POSTER -3

Transcript profiling of cytokinin action in *Arabidopsis* roots and shoots discovers organ-specific responses

Wolfram G. Brenner (1), Thomas Schmülling (1)

(1) FU Berlin, Dahlem Centre of Plant Sciences

Cytokinin regulates root and shoot growth in opposite ways: In shoots, it induces growth; in roots, it inhibits growth. Little is known about the assumed organ-specific regulation of gene expression involved in these differential activities. To get more insight into transcript regulation triggered by cytokinin in roots and shoots, we studied genome-wide gene expression in cytokinin-treated and cytokinin-deficient roots and shoots.We found by principal component analysis that the immediate-early response to cytokinin differs from the later response, and that the transcriptome of cytokinin-deficient plants is different from both the early and the late cytokinin induction response. A higher cytokinin status in the roots activated the expression of numerous shoot-specific genes, while a lower cytokinin status in the shoot repressed the expression of shoot-specific genes. This shift mostly affected nuclear genes encoding plastid proteins and indicated a cytokinin influence on the organ-specific transcriptome pattern independent of morphological organ identity.Novel cytokinin-regulated genes and new insights into the activities of cytokinin, including crosstalk with other hormones and translational control were found, which had escaped earlier discovery due to unspecific sampling.

S27 - POSTER -4

Characterization of CKX1-interacting HIPP proteins

Henriette Weber (1), Tomáš Werner (1)

(1) Freie Universität Berlin

The plant hormone cytokinin is an essential regulator of many physiological and developmental processes in plants. The concentration of cytokinin is effectively controlled through oxidative degradation catalyzed by cytokinin oxidase/dehydrogenase (CKX) enzymes. In order to understand the molecular mechanisms underlying the activity of CKX proteins, one of our approaches has been to seek for CKX-interacting proteins which could modulate the CKX enzyme activity, its subcellular localization or stability, or mediate a cellular activity primarily unrelated to CKX metabolic function. In a yeast two-hybrid screen, we found several HIPP proteins to interact with CKX1. The *Arabidopsis* HIPP protein family comprises 48 members and is defined by the occurrence of a heavy metal-associated domain (HMA) and an isoprenylation motif; a motif combination which is plant-specific. The function of HIPP proteins is currently unknown. Here, we present first results of our efforts to characterize the function of isolated HIPP proteins, including mapping of the interacting regions, determination of subcellular localization, and characterization of HIPP-overexpressing plants. The relevance of HIPP proteins for CKX activity and plant cytokinin responses will be discussed.

S27 - POSTER -9

Isolation and characterization of new point mutants of AUXIN BINDING PROTEIN1 (ABP1)

Yunus Effendi (1), Günther F.E. Scherer (2)

(1) Molekulare Ertragsphysiologie - Leibniz Universität Hannover; (2) Herrenhäuser Str. 2, D30419 Hannover, scherer@zier.uni-hannover.de

We showed that the heterozygous knockout mutant *abp1/ABP1* has defects in auxin physiology-related responses and lower transcript levels of early auxin-regulated genes (Effendi et al., 2011, Plant J. 65, 282).We designed two mutants, M7 and M8, by introducing a mutated cDNA, coupled to *35S* promoter, into heterozygous *abp1/ABP1* plants and screened for null wt gene transcription in the progeny. We also isolated transgenic plants expressing wt *ABP1* cDNA coupled to 35S (ABP1-OEX). M7 and M8 produced slightly shorter main roots but fewer lateral roots in response to auxin. They showed slowed hypocotyl phototropism and slowed root and hypocotyl gravitropism, which the ABP1-OEX did not show. M7 and M8 flowered early in SD but not ABP1-OEX. We also investigated the *abp1-5* (H94>Y94) point mutant. *abp1-5* shows slowed root gravitropism but hypocotyl phototropism or gravitropism was not changed and it flowered at the same time as Col in SD. qPCR of auxin-induced gene regulation in *abp1-5* shows it is a weak allele and first results on M7 and M8 show disturbed regulation of auxin-regulated genes. *ABP1* point mutants will be a valuable tool in auxin research.

S27 - POSTER -10

The role of cytokinin in regulating root system architecture in *Arabidopsis thaliana*

Ling Chang (1), Tanja Rublack (1), Eswar Ramireddy (1), Thomas Schmülling (1)

(1) Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences, Freie Universität Berlin

Plant roots are responsible for nutrient and water uptake and provide physical support to the plant. Lateral roots (LR) make a considerable contribution to the root architecture and originate postembryonically, which is regulated by hormones and environmental signals. Cytokinin is a negative regulator of LR formation but the mechanisms of its action on LR development and its role in modulating root system architecture in response to environmental signals is still unclear.Phenotypic analysis of roots from cytokinin-deficient plants showed that most of them have increased LR density and altered distribution of LR primordia. In addition, analysis by VisuaLRTC revealed that expression of *CKX1*, *IPT3*, *IPT5*, *AHK2*, *AHK3* and *CRE1/AHK4* changed significantly during LR initiation and development. In order to get a first insight in the interplay with other factors regulating LR formation and growth, the root system of plants with an altered cytokinin status was analyzed on media containing various other hormones or soil-borne nutrients such as nitrogen and phosphate. The preliminary results showed that the cytokinin system is tightly interconnected with other signaling systems and that the cytokinin status predetermines the response to a number of different factors.

S27 - POSTER -11

Identification of cis-regulatory elements for gene regulation in response to cytokinin

Eswar Ramireddy (1), Andreas Pfeifer (2), Wolfram Brenner (1), Alexander Heyl (1), Thomas Schmülling (1)

(1) Dahlem Centre of Plant Sciences, Freie Universität Berlin; (2) Dahlem Centre of Plant Sciences, Freie Universität Berlin,

The identification of functional *cis*-acting DNA regulatory elements is a crucial step towards understanding the regulation of gene expression. In *Arabidopsis*, 11 B-type response regulators (B-type ARRs) regulate the transcription of their target genes in response to cytokinin. In fact, using the SRDX chimeric repressor technology, it was shown that B-type ARRs mediate most if not all of the transcriptional response to cytokinin. The B-type ARRs tested so far bind *in vitro* optimally to the core DNA sequence 5'-(A/G) GAT(T/C)-3'. However, so far the relevance of this *in vitro* binding sequence has not yet been demonstrated *in planta*. In the present study we attempted to identify specific target genes of one of the B-type ARR, ARR1, and the functionally relevant *cis*-acting element(s) for the cytokinin response. To this end, transcription profiles of wild-type, *arr1* mutant and *35S:ARR1* transgenic seedlings were compared and 24 genes were identified as putative specific target genes of ARR1. The promoter of one target gene (*ARR6*) was analysed in more detail by deletion analysis. The results not only confirm for the first time the functionality of above mentioned element *in planta* but also identified a novel *cis*-acting promoter region that functions co-operatively with the core element.

S27 - POSTER -12

Methyl-salicylate is a mobile form of salicylic acid in *Arabidopsis thaliana* infected by *Plasmodiophora brassicae*

Ivana Sola (1), Gordana Rusak (1), Jutta Ludwig-Müller (2)

(1) Faculty of Science; (2) Technische Universität Dresden

The mobile signals for systemic acquired resistance (SAR) in plants are plant-pathogen specific. It is known that salicylic acid (SA) enables the establishment of SAR in plants infected by biotrophic pathogens, but the nature of the long-distance mobile signal for SAR depends on the plant-pathogen system. The clubroot disease is one of the most devastating diseases affecting all the members within the plant family *Brassicaceae* by causing serious losses of vegetable crops worldwide. In this study, we wanted to investigate whether methyl-salicylate (MeSA) is a mobile form of SA implicated in this plant-pathogen interaction. We have chosen *Arabidopsis thaliana* as a host organism for *Plasmodiophora brassicae* – the causal agent of clubroot, because the infection process is fast and the resistance of *Arabidopsis* to *P. brassicae* is conferred by a small number of genes. Using a GC-MS method, we monitored the transport and the metabolism of exogenously applied deuterated salicylic acid and its derivative, deuterated methyl-salicylate, through the whole plant of healthy and *P. brassicae*-infected *Arabidopsis*. The results showed that MeSA is a mobile form of SA in *Arabidopsis* clubroots.

Isolation and Characterization of New Point Mutants of AUXIN BINDING PROTEIN 1

Yunus Effendi and Günther F.E. Scherer
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Abstract

We show that the heterozygous abp1/ABP1 has defect in auxin physiology-related responses and lower transcript level of early auxin-regulated genes (Effendi, et al., 2011. Plant J. 65:285). We designed two mutants, M7 and M8, by introducing a mutated ABP1 cDNA, coupled to 35S promoter, into heterozygous abp1/ABP1 plants and screened for null WT gene transcription in the progeny. We also isolated transgenic plant expressing WT ABP1 cDNA coupled to 35S (ABP1-OEX). M7 and M8 produced slightly shorter main root but fewer lateral root, in response to auxin. They showed slowed hypocotyl phototropism and slowed root and hypocotyl gravitropism, which ABP1-OEX did not show. M7 and M8 flowered early in SD but not ABP1-OEX. We also investigated the abp1-5 (H94>T94) point mutant. abp1-5 showed slowed root gravitropism but hypocotyl phototropism or gravitropism was not changed and it flowered at the same time as Col-0 in SD. ABP1 point mutants will be a valuable tool in auxin research.

Keyword: ABP1, mutants, auxin-inuced genes

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, 2010a,b). ABP1-mediated rapid responses such as membrane hyperpolarization, channel regulation, proton extrusion, phospholipase A activation (Scherer and Andrè, 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 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+-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 (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, 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 thesteady-state level of ABP1 and auxin transport over a time course consisent 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., 001b) 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–2.s –1 (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–2.s –1) 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 (ZeissGermany). 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 (12/12h) days. For the auxin the medium was removed and replaced by fresh medium containing 10 μM 1-NAA. Seedlings were blotted on filter paper and frozen in liquid nitrogen for further use. For quantitative RT-PCR, 4 μg of total RNA was prepared with the NucleoSpin® RNA Plant kit according to the manufacturer's instructions (Macherey and Nagel) and transcribed to first strand cDNA with RevertAidTM H Minus First Strand cDNA Synthesis kit (Fermentas). Primers and methods were as described previously (Effendi et al., 2011; Effendi and Scherer, 2011). 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 ΔΔCt method using the equation: relative expression=2–[ΔCtsample–ΔCtcontrol], with ΔCt=Ctsample gene–Ctreference gene, where Ct refers to the threshold cycle determined for each gene in the early exponential amplification phase (Livak and Schmittgen, 2001). The control treatment at t=0min was set as 1-fold expression level. For statistical analysis the REST 2008 software (Pfaffl et al., 2002) was sed.

RESULT

The mutant abp1-5 containing a histidine 94→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 (Fig. 1d) confirming that ABP1 affects auxin function(s).

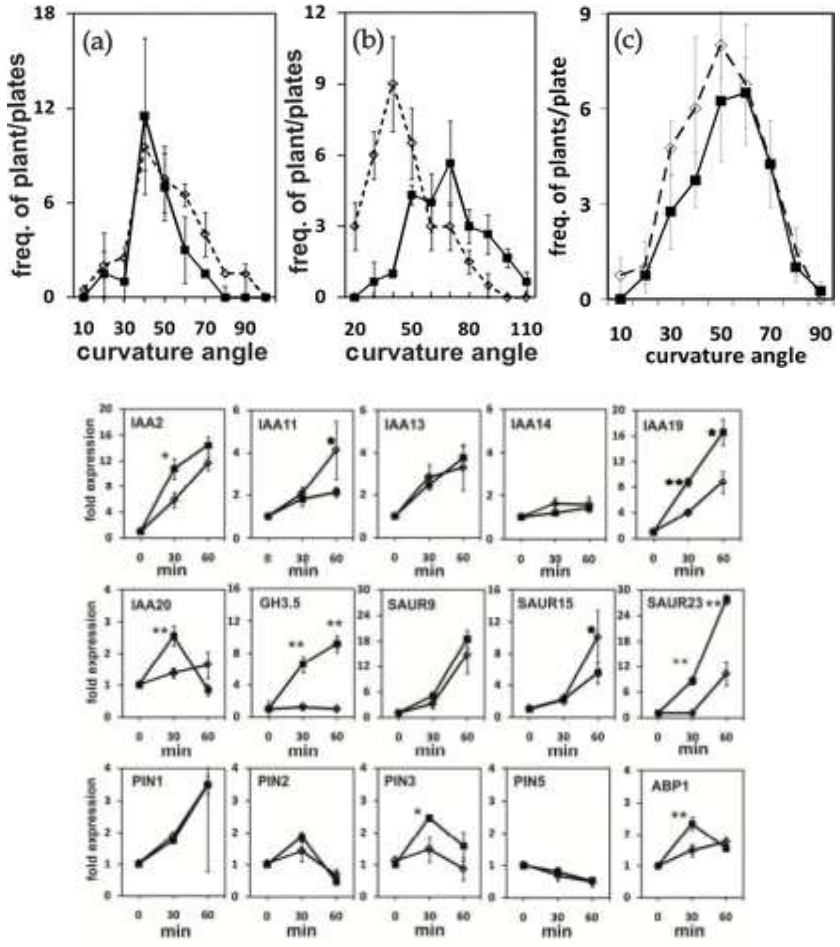

Fig.1. Gravitropic and phototropic responses in 3-d-old dark-grown Col-0 (black squares) and abp1-5 (diamonds) seedlings. (a) Gravitropic bending angles of hypocotyls after 24h tilting by 90° (mean Col: 44.8°; n=57; mean abp1-5: 46.7°; n=42; P <0.54; difference not significant). (b) Gravitropic bending angles of roots after 24h 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 8h lateral blue light (10 µmol 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. (d) Rapid regulation of early auxin genes by 10 µM 1-NAA in Col-0 wild type and abp1-5 mutant seedlings.

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.

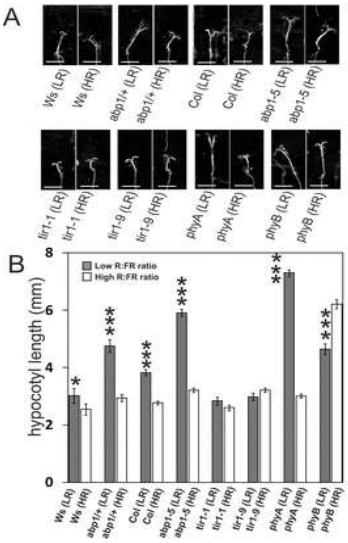

Fig.2. Shade-avoidance responses in abp1-5 and abp1/ABP1 compared with Col, phyA-211, and phyB-9. Shade avoidance was tested by growing seedlings for 3 d in WL and for 3 more days in WL or white plus added low R:FR ratios (LR, simulated shade) or high ratios of R:FR (HR, non-shade). Seedlings from seeds 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=5mm. (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).

TIR1 regulates gene transcription by auxin-stimulated ubiquitination of AUX/IAA proteins which are negative cotranscription 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 Pr 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. Greater NPA inhibition was simply associated with taller hypocotyls, a sensitivity difference in mutants or wild types to NPA concentration was small if any.

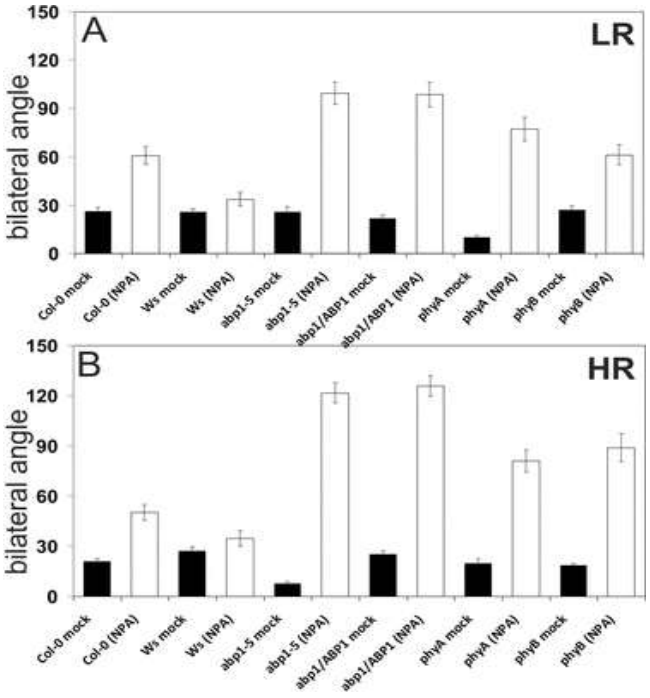

Fig.3. Diagnostic effects of 5 μM NPA on hypocotyl gravitropic orientation in (A) low and (B) high ratio R:FR light in abp1-5 and abp1/ABP1 and phytochrome mutants and wild types. Data are from 24 to 54 seedlings per assay (SE). The genotype of abp1/ABP1 plants was verified by PCR. In LR Col and phyB 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).

To test the hypothesis that ABP1 is involved in the response, the expression of shade-induced marker genes was quantified after 1h 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, HFR1) 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; Kunihiro 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 1h 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 1h 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.

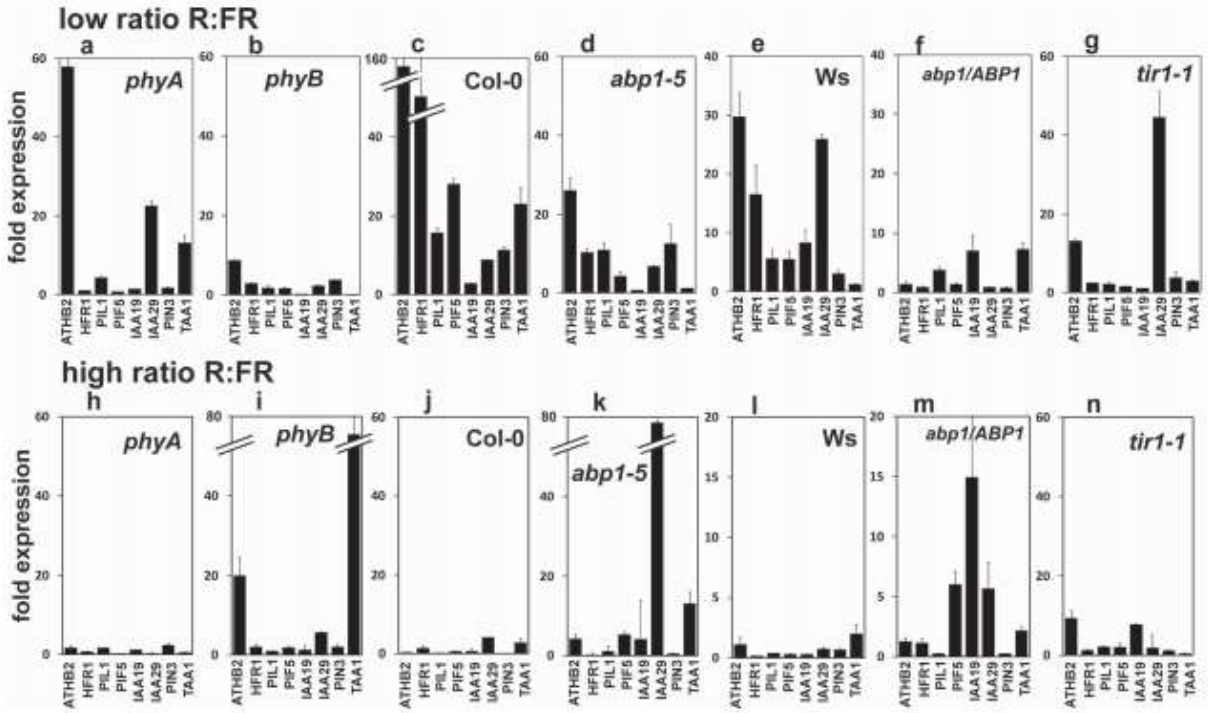

Fig.4. Comparison of regulation of genes by low ratio R:FR (a–f, shade) and high ratio R:FR (g–l, non-shade) in Col, phyA, phyB, abp1-5, and tir1-1. Seedlings were tested by growing for 3 d in WL and for 1h in WL or white plus added low R:FR ratios or high ratios of R:FR. Expression was normalized to 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.

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×) compared with Col, phyA, or tir1 but still high in abp1-5 (15×) 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; Kunihiro et al., 2010). Our physiological results and our results on auxin-induced gene expression (Fig. 5) 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 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.

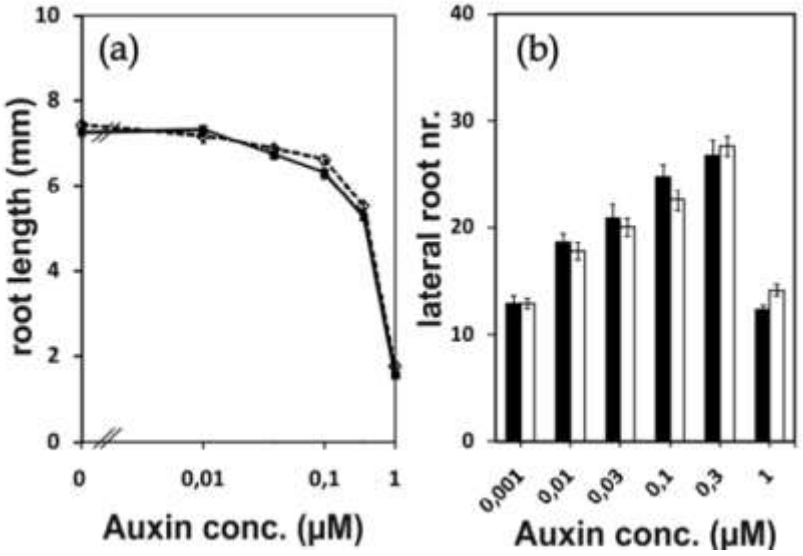

Fig.5. Auxin sensitivity of *abp1-5*. (a) Root length of 10 days old light-grown Col-0 (black squares) and *abp1-5* (diamonds) seedlings. (S.E., n=30). (b) Lateral root formation in response to 1-NAA in 10 days old light-grown Col-0 (black bars) and *abp1-5* (white bars) seedlings. (S.E., n=30).

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 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 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.

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Isolation and Characterization of New Point Mutants of AUXIN BINDING PROTEIN 1

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Abstract

We show that the heterozygous *abp1/ABP1* has defect in auxin physiology-related responses and lower transcript level of early auxin-regulated genes (Effendi, *et al.*, 2011. Plant J. 65:285). We designed two mutants, M7 and M8, by introducing a mutated ABP1 cDNA, coupled to 35S promoter, into heterozygous *abp1/ABP1* plants and screened for null WT gene transcription in the progeny. We also isolated transgenic plant expressing WT ABP1 cDNA coupled to 35S (ABP1-OEX). M7 and M8 produced slightly shoter main root but fewer lateral root, in response to auxin. They showed slowed hypocotyl phototropism and slowed root and hypocotyl gravitropism, which ABP1-OEX did not show. M7 and M8 flowered early in SD but not ABP1-OEX. We also investigated the *abp1-5* (H94>T94) point mutant. *abp1-5* showed slowed root gravitropism but hypocotyl phototropism or gravitropism was not changed and it flowered at the same time as Col-0 in SD. *ABP1* point mutants will be a valuable tool in auxin research.

Point mutants M7 and M8 showed defects in response to auxin and less sensitive in regulation of early auxin-regulated genes

Early flowered in M7 and M8

Fig. 1. Phenotypes of the 43 days old LD-grown plants (A) Mutants M7 and M8 flowered earlier in comparison to OEX-ABP1 and wt. Statistic difference of flowering time is showed in the fig (B). M7 and M8 produced fewer rosette (fig.C) but have more shoots in comparison to wt (fig.1D) which indicate a defect in apical dominance. Asterix indicates significantly difference ($p < 0.001$, in A and B; $p < 0.05$ in D) relative to OEX-ABP1.

Lower number of lateral root in response to different auxin concentration in mutant M7 and M8

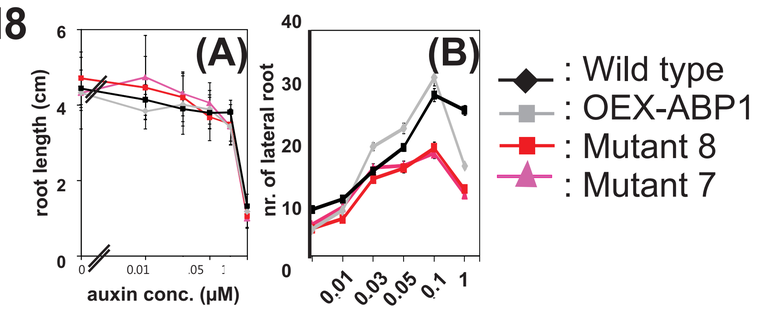


Fig. 2. Auxin sensitivity. (A) No difference in hypocotyls length was observed between M7, M8 to OEX-ABP1 or WT in response to different auxin concentrations (0.001µM, 0.01µM, 0.02µM, 0.03µM, 0,1µM,0.3µM, and 1µM) (B). However, M7 and M8 produced lower number of lateral root in comparison to OEX-ABP1 and wt. Plants were grown on agar plates for 4 days under normal light before were transferd to auxin-contained agar for 3 days. (for each auxin concentration n: 30)

Less response to gravitropism and phototropism in mutants M7 and M8

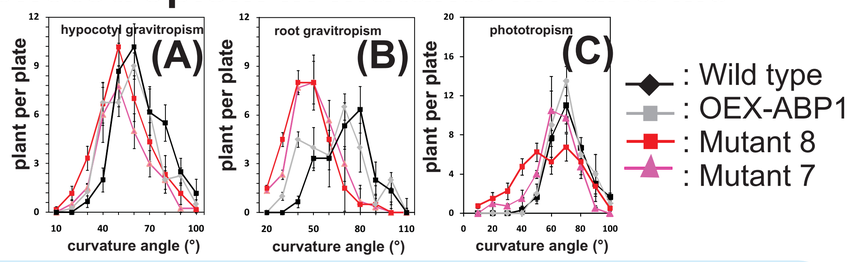


Fig.3. Response of hypocotyls and/or roots to gravity and light of mutants M7 and M8 seedlings. (A) The gravitropic response of hypocotyls of M7 and M8 showed lower responses to gravity by producing bending angles peak at 50°, whereas OEX-ABP1 and wt are 60°.(B) Similar response was observed on root gravitropism where M7 and M8 respond smaller to gravity in comparison to OEX-ABP1 and wt. 4 day dark-grown seedlings were grown on agar plates and were tilted 90° 24 h. (C) M7 and M8 produced smaller bending angles in response to 8 h 10µmol $m^{-2}s^{-1}$ lateral blue light in comparison to OEX-ABP1 and wt. Plants were grown 3 days on dark before applied with 10µmol $m^{-2}s^{-1}$ of lateral blue light.

Lower transcript abundance of early auxin-regulated genes and PIN genes in M7 and M8

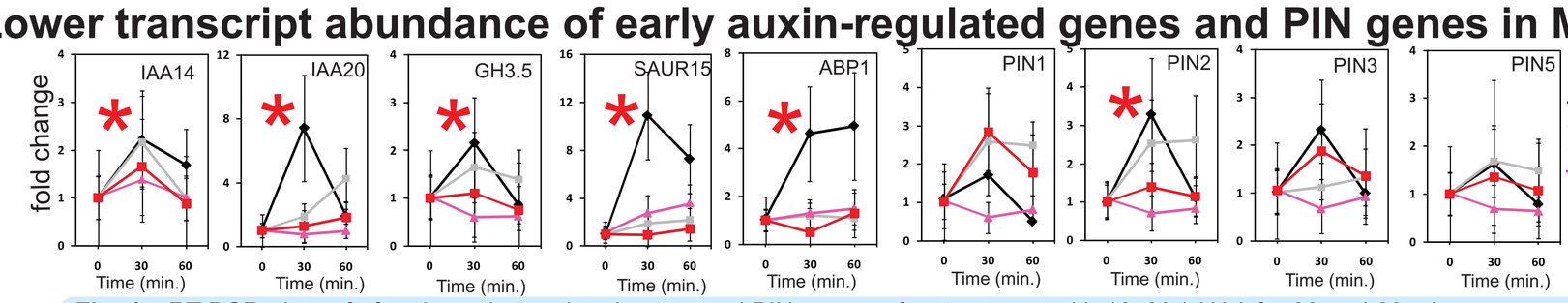
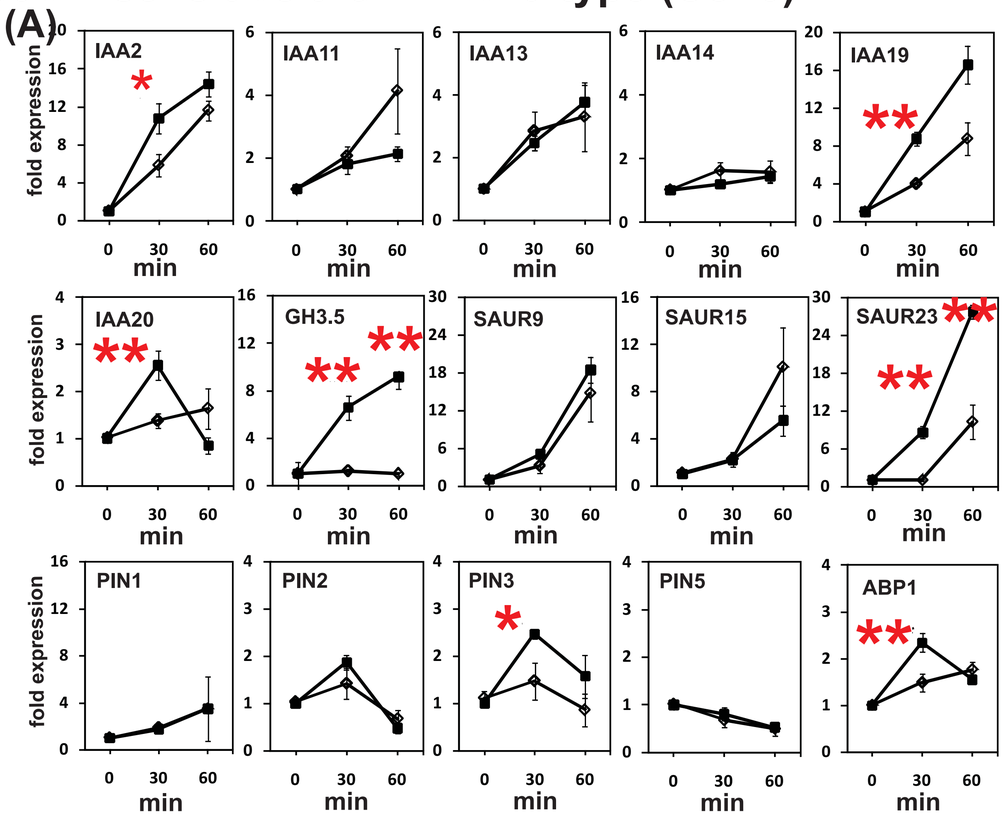


Fig. 4. qRT PCR data of of early auxin-regulated genes and PIN genes after treatment with 10µM 1-NAA for 30 and 60 min. M7 and M8 showed lower response in the expression of *GH3.5* and *PIN2* genes after 30 min auxin application in comparison to OEX ABP1 and wt. In comparison to wt only, most of the genes (*IAA14, IAA20, GH3.5, SAUR15, ABP1,* and *PIN2*) were lower regulated after 30 min 1-NAA application in M7 and M8. Data was collected from three biological replications and three technical replications of each.

Even a weak point mutant *abp1-5* showed slightly defects in auxin-regulated characters and lower response to auxin in regulation of early auxin-regulated genes

Transcriptional regulation of some early auxin-response genes and PIN genes in *abp1-5* mutant was slightly less auxin sensitive than in wild type (Col-0)



Phenotype appearance of *abp1-5* mutant

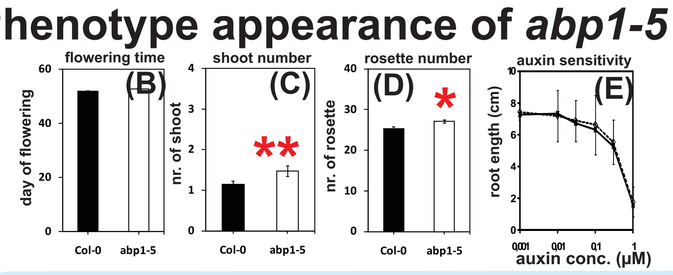


Fig. 5. Phenotypic appearance of 52 days old LD-grown plants. (A). Representative picture of 52 days old plants showed no difference in flowering time between *abp1-5* mutant and Col-0. (B) Graphic quantification of flowering time. Plants were grown under LD condition. (C) *abp1-5* mutant produced more shoots and rosette leaves (D) in comparison to Col-0. (E) No difference in root length was observed between *abp1-5* and Col-0 in response to different auxin concentrations.

Slightly defects in auxin-regulated phenotypes of *abp1-5* mutant

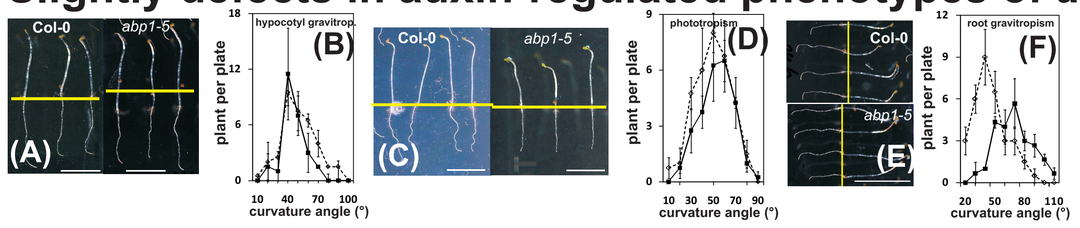


Fig. 6. Responses to gravity and light in *abp1-5* mutant seedlings (A) Representative images of a 3 d old seedlings after 24 gravitropic response and (B) their graphic quantification of hypocotyl angle showed no difference. (C) Representative image of 3 d dark-grown seedlings in responses to 8 h phototropism (10µmol $m^{-2}s^{-1}$ blue light) and (D) their quantification of hypocotyls bending which showed *abp1-5* produced smaller angles in comparison to Col-0. (E) Representative image of 3 d dark-grown seedlings after 24 h gravitropic response and (F) their grapic quantification which showed lower response in *abp1-5* in comparison to Col-0. Data were collected from three independent replications (n= 40-90 of each). Open diamonds: *abp1-5* mutant, black square: Col-0.

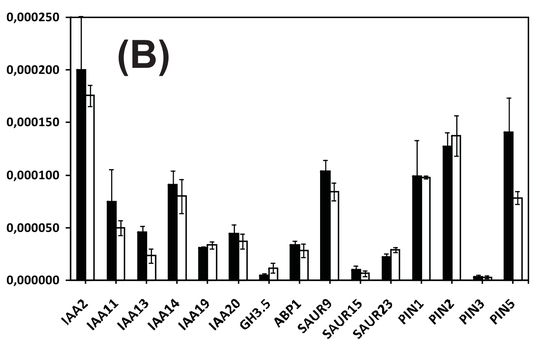


Fig. 7. Less auxin sensitive in transcriptional regulation of some early auxin-response genes and PIN genes in *abp1-5* mutant in comparison to wt in response to 10µM 1-NAA at 3 time points (0, 30, 60 min). A. Five genes (*IAA2, IAA19, IAA20, GH3.5, SAUR23*) of 10 early auxin-response genes were observed less regulated in *abp1-5* mutant in comparison to wt, whereas only *PIN3* of 4 PIN genes which was observed less regulated. Data was collected from 2-3 independent biological replication and 3 technical replication of each. Asterix indicates statistically significant ($p<0.001$ or $p<0.05$). Eventhough, basal level of transcriptional abundants the most of investigated-genes in *abp1-5* and wt were similiar, no statistic difference (B).

Conclusion: Our ABP1 point mutants, M7 and M8, showed defect in auxin-regulated phenotypes and low response to auxin in comparison to OEX-ABP1 and wt. A weak point mutant *abp1-5* also showed slightly similar response to auxin. All together it gives significant proves that a mutant of ABP1 will be a good tool for further researches in auxin physiology, especially auxin signaling.

Reference: **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. Plant. J 65: 282-294.

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