IPGSA CONFERENCE 2010

20th International Conference on Plant Growth Substances

28th June to 2nd July 2010
Tarragona (SPAIN)

I309

CERTIFICATE OF ATTENDANCE

We hereby certify that,

MR. YUNUS EFFENDI

has attended the

20th International Conference on Plant Growth Substances

held at the Universitat Rovira i Virgili, Tarragona (Spain), on 28th June to 2 July 2010.

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	June 28 (Monday)	June 29 (Tuesday)	June 30 (Wednesday)	July 1 (Thursday)	July (Friday)
09:00	Registration (opens every day)	**P2** Hormone Biosynthesis and Transport *Shinjiro Yamaguchi (Japan)*	**P3** Hormone Perception and Signaling *Joe Kieber (USA)*	**PS09** Evolution of Plant Hormone Signaling **PS10** Root Development **PS11** Abiotic Stress **PS12** Chemical Biology	**P4** Hormones and Environment *Salomé Prat (Spain)*
09;45		*Paul Staswick (USA)*	*Bonnie Bartel (USA)*		*Moto Ashikari (Japan)*
10:30		Coffee break	Coffee break	Coffee break	Coffee break
11:00		*Tetsuya Higashiyama (Japan)*	*Zhiyong Wang (USA)*	**PS09** Evolution of Plant Hormone Signaling **PS10** Root Development **PS11** Abiotic Stress **PS12** Chemical Biology	Steve Penfield (UK)
11:30	Opening				
11:45	Silver Medal Lectures *Joanne Chory (USA)*	*Jiri Friml (Belgium)*	*Erwin Grill (Germany)*		*Luis Herrera-Estrella (Mexico)*
12:30	*Tokao Yolota (Japan)*	Lunch & Posters	Lunch & Posters	Lunch & Posters	Closing
13:00					Farewell Lunch
13:15	*Mark Estelle (USA)*				
14:00	Lunch				
15:00	**P1** Hormones and Development *Ottoline Leyser (UK)*	**PS01** Hormone Biosynthesis **PS02** Seed Development and Germination **PS03** Auxin Transport **PS04** Light Responses	**PS05** Hormone Perception and Signaling **PS06** Shoot Development **PS07** Defense responses **PS08** Systems Biology	**PS13** Hormone Interactions **PS14** Reproductive Development **PS15** Hormones and Biotechnology **PS16** Epigenetics/Small RNAs	
15:45	*Eliezer Lifschitz (Israel)*				
16:30	Coffee break	Coffee break	Coffee break	Coffee break	
17:00	*Markus Grebe (Sweden)*	**PS01** Hormone Biosynthesis **PS02** Seed Development and Germination **PS03** Auxin Transport **PS04** Light Responses	**PS05** Hormone Perception and Signaling **PS06** Shoot Development **PS07** Defense responses **PS08** Systems Biology	**PS13** Hormone Interactions **PS14** Reproductive Development **PS15** Hormones and Biotechnology **PS16** Epigenetics/Small RNAs	
17:45	*Veronica Grieneisen (UK)*				
18:30	Wellcome Reception	Tarragona Roman Ruins visit		IPGSA Business Meeting	
19:30					
20:00			congress Dinner		

PS05-18 **STRUCTURAL BASIS FOR GIBBERELLIN RECOGNITION BY ITS RECEPTOR GID1**
Miyako Ueguchi-Tanaka (Japan)

PS05-19 **MIS-REGULATION OF EARLY AUXIN-INDUCED GENES IN PHOSPHOLIPASE A KNOCKOUTS**
Corinna Labusch (Germany)

PS05-20 **CHEMICAL SCREENING AND CHARACTERIZATION OF INHIBITORS FOR GIBBERELLIN RECEPTOR**
Jung-min Yoon (Japan)

PS05-21 **A POSSIBLE CYTOSOLIC ACTIVITY FOR THE GA RECEPTOR, GID1**
Sivan Sagiv (Israel)

PS05-22 **DOMAIN REQUIREMENTS FOR DIFFERENT ROLES OF THE ETR1 ETHYLENE RECEPTOR**
Brad Binder (USA)

PS05-23 **TRANSCRIPT PROFILING OF CYTOKININ ACTION IN ARABIDOPSIS ROOTS AND SHOOTS**
Brenner Wolfram (Germany)

PS05-24 **THE IMA GENE ENCODES AN ADAPTER MINI ZINC FINGER PROTEIN REGULATING THE DEVELOPMENT OF FLORAL MERISTEM IN TOMATO.**
LEBLOND JULIE (FRANCE)

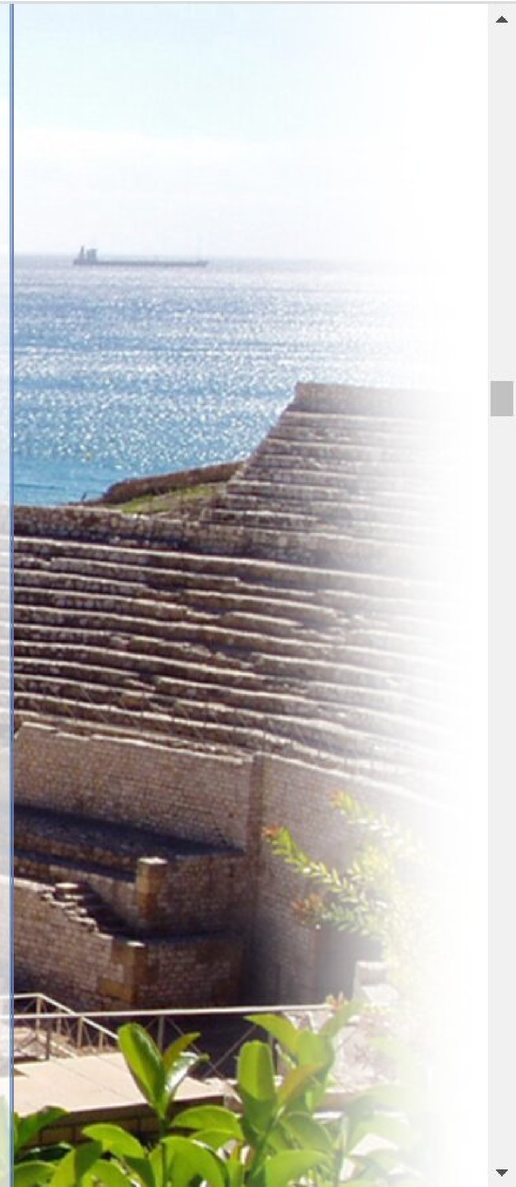
PS05-25 **QUANTITATIVE DIFFERENTIAL ANALYSIS OF PLANT PROTEOME IN A RESPONSE TO HORMONAL REGULATIONS**
Marketa Zdarska (Czech Republic)

PS05-26 **DYNAMIC STRUCTURE AND BINDING SPECIFICITY OF THE RECEIVER DOMAIN OF SENSOR HISTIDINE KINASE CKI1**
Tomas Klumpler (Czech Republic)

PS05-27 **ATPLA-I, A PHOSPHOLIPASE A WITH FUNCTIONS IN GRAVITROPISM, PHOTOTROPISM AND ROOT TIP MOVEMENTS**
Günther Scherer F.E. (Germany)

PS05-28 **AUXIN-BINDING PROTEIN1 (ABP1), THE SECOND AUXIN RECEPTOR**
Yunus Effendi (Germany)

PS05-29 **MUTATION OF TRNA SPECIFIC ISOPENTENYLTRANSFERASES AFFECTS ARABIDOPSIS PHENOTYPE**
Silvia Gajdosova (Czech Republic)



Auxin-binding protein1 (ABP1), the second auxin receptor

Yunus Effendi[1] , Steffen Rietz[2] , Urs Fischer[3] , Günther F.E. Scherer [1]
[1]Molecular Developmental Physiology, University Hannover, Herrenhäuser Str. 2, D-30419 Hannover, Germany;
[2]Max Planck Institute for Plant Breeding Research, Cologne;3) Forstbotanik und Baumphysiologie University Göttingen.
Scherer@zier.uni-hannover.de

Abstract

Despite of knowing the 3-dimensional structure ABP1 is not fully acknowledged as an auxin receptor.We used the homozygous lethal ABP1 insertional mutant (Chen et al. 2001) which is viable in the heterozygous abp1/ABP1 state. abp1/ABP1 seedlings are defect in phototropism and gravitropism of roots and shoots. Those populations are composed of a major slow reacting and a minor normal reacting group. abp1/ABP1 seedlings show strong root slanting, longer hypocotyls, and only slightly increased lateral root numbers. Root auxin responses (lateral roots,main root length) in abp1/ABP1 seedlings are only slightly less sensitive than in wt. In short and long days abp1/ABP1 plants flower earlier. They have more branches and decreased main stem diameter, indicating decreased apical dominance. Auxin-induced genes (qPCR of: IAA2, IAA11, IAA12, IAA13, IAA14, IAA19, IAA20, SAUR9, SAUR15, SAUR23, GH3.5, ABP1) respond to auxin (0.1µM/1µM/10µM) 2-10-fold stronger in wt than in abp1/ABP1 seedlings (30 & 60 min). Auxin content and uptake of auxin in abp1/ABP1 seedlings is not distinguishable from wt. Basipetal auxin transport in abp1/ABP1 roots is slower than in wt. Thus ABP1 is a receptor with probable functions in auxin transport and gene regulation. The necessary functional link to TIR1-linked gene regulation could be provided by phospholipase(s) A (FEBS Lett. 2007, 581:4205-4211)

Keyword: ABP1, auxin-induced genes, gravitropism, phototropism

Introduction

AUXIN-BINDING PROTEIN 1 (ABP1) was the first protein described as having specific auxin-binding activity (Napier et al., 2002). Previously, auxin-binding activity, probably resulting from ABP1, had been reported for membranes isolated from maize coleoptiles (Hertel et al., 1972). Initially, research on ABP1 functions focused on rapid regulation of membrane potential and potassium channels (BarbierBrygoo et al., 1989, 1991; Thiel et al., 1993). Clear evidence of a link to typical auxin functions such as cell elongation, cell division or lateral root formation was lacking at first, as no ABP1 mutants or antisense plants were available. The Arabidopsis thaliana genome contains only one ABP1 gene, and its knockdown resulted in embryo lethality of homozygous progeny (Chen et al., 2001b). Although the embryo lethality of the Arabidopsis homozygous abp1 knockout mutant demonstrated the functional importance of ABP1, it hindered investigations on the post-embryonic functions of ABP1. Determination of the 3D structure of ABP1 revealed a specific binding site for auxins (Woo et al., 2002). Moreover, ABP1 is a small glycoprotein that is abundant in the ER, with only a small proportion exposed on the outer leaflet of the plasma

membrane (Napier et al., 2002). As ABP1 has no transmembrane domain, a docking protein was postulated to exist that linked auxin perception to intracellular signaling (Kla¨mbt, 1990). However, no such membrane anchor for ABP1 has yet been identified. The study showed that ABP1 functions as an extra-cytoplasmic protein and that ABP1 inhibition hinders the cell cycle at the G1/S and G2/M phase transitions. This concept of suppression of ABP1 function by antibody binding was expanded by ethanol-controlled expression of the antibody in planta (Braun et al., 2008; Tromas et al., 2009). Suppression of ABP1 function by ethanol-stimulated antibody expression inhibited both cell expansion and cell division in these plants. Moreover, expression of the anti-ABP1 antibody for 8 h also led to down-regulation of several IAA genes, suggesting that ABP1 also functions in auxin-induced gene regulation, which, at that time, was attributed exclusively to TIR1 and its homologs (Mockaitis and Estelle, 2008). We previously showed that auxin activates phospholipase A, and that inhibitors of phospholipase A inhibited hypocotyl elongation and up-regulation of early auxininduced genes (Paul et al., 1998; Scherer et al., 2007), although the inhibitors did not directly affect TIR1 activity. In this way, we provided indirect evidence that an auxin receptor other than TIR1 participates in gene regulation of auxin-induced genes. In an ongoing study to generate viable mutants of ABP1 in order to provide a 'missing link' between the lethal abp1 knockouts and the wild-type, we performed experiments on heterozygous abp1/ABP1 plants. The heterozygous plants are viable, whereas homozygosity leads to embryo lethality (Chen et al., 2001b). Surprisingly, heterozygous plants showed physiological and morphological features that clearly deviated from wild-type. In addition, even as early as 30 min after auxin challenge, a number of IAA genes and other early auxin-regulated genes were up-regulated to a much lower extent in abp1/ABP1 seedlings compared to wild-type seedlings.

EXPERIMENTAL PROCEDURES

Plant material

Hetozygous abp1/ABP1 mutant seeds (stock number N6489) were obtained from the Nottingham Arabidopsis Stock Center (http://arabidopsis.info/), and these proved to be kanamycin-resistant. For long- or short-day experiments, seedlings were not selected on kanamycin agar but were sown directly on peat-based compost soil (Einheitserde, http://www.einheitserde.de/) containing 30% silica sand. The genotypes were determined by PCR. Seeds from abp1/ABP1 plants were sown on kanamycin-containing medium and transferred to kanamycin-free medium after 4 days of growth on upright agar plates. Afterwards, they were selected according to their slanting angle. The experiments shown in Figures 1(a,b) and 2 were performed this way on upright agar plates. In the experiments shown in Figure 1(c–e), all seedlings were planted on kanamycin-free medium and the results confirmed the segregation of 2:1 abp1/ABP1 to wild-type (Chen et al., 2001b). For quantifications, seedlings were scanned using a CanonScan 8800F (resolution of 600 dots per inch; Canon, http://www.canon-europe.com). Root lengths and angles were measured using AXIOVISIO LE version 4.6 software (Zeiss, http://www.zeiss.com/).

For transcription measurements and auxin uptake experiments (Figures 5 and 6), seedlings were grown in half-strength MS agar medium for 14 days under long-day conditions, the wild-type without kanamycin, and seeds from a kanamycin resistant abp1/ABP1 plant in medium containing 50 lg ml)1 kanamycin. Then resistant abp1/ABP1 seedlings were selected, washed three times for 5 min in medium without kanamycin, and grown for a further 5 days in half-strength MS liquid medium without kanamycin. Wildtype seedlings were treated the same way, but without kanamycin. For auxin treatment, the medium was removed and replaced by fresh medium without

or with the IAA concentration indicated. Seedlings were blotted on filter paper and frozen in liquid nitrogen for further use.

IAA uptake measurements

For auxin uptake experiments, treated seedlings were quickly washed five times in sterile water, blotted and frozen in liquid nitrogen in 200 mg aliquots. To each sample, 1 ml of CHCl3/CH3OH/H2O (1:2:0.3) containing 40 pmol deuterated IAA (d2-IAA) (Sigma,http://www.sigmaaldrich.com/) was added as an internal standard, and the mixture was shaken for 10 min at 70C. Following centrifugation (18 000 g, 4C, 5 min), the supernatant was collected, and the sediment was re-extracted with 0.5 ml CHCl3/CH3OH (1:2) and pooled with the previous extract. Phase separation was induced by addition of 0.5 ml H2O, and, after vortexing for 6 sec, samples were kept at)20C for 30 min. After brief centrifugation, the upper phase was collected and reduced to approximately 250–300 ll in a Speedvac concentrator (Eppendorf, http://thermoscientific.com). The samples were acidified with 300 ll 0.2% trichlorofluoric acid, and extracted twice with 600 ll ethyl acetate/hexane (3:1) by vigorous vortexing for 30 sec. The organic phases were transferred to a glass vial and reduced to complete dryness in a Speedvac concentrator. Samples were derivatized using 80 ll N-methyl-N-(trimethylsilyl)trifluoroacetamide (pyridine salt) with 1% trimethylchlorosilane (Fluka, http://www.sigmaaldrich.com) (1:1) for 30 min at 90C then overnight at room temperature. The analysis was performed using a quadrupole GC-MS system (Agilent, http://www.agilent.com) by injection of 1 ll at an injector temperature of 250C. With a split ratio of 1:1, the sample was loaded onto an HP-5MS column (Agilent) at 1.5 ml min)1. The oven temperature was set to 100C for 2 min, and gradually increased by 10C per minute to 160C, 3C per minute to 193C and 12C per minute to 300C, and held for 3 min. Identification of IAA and d2-IAA was based on retention times and fragmentation patterns. Ions were detected by selected ion monitoring and quantified using ions m/z 202 (IAA) and 204 (d2-IAA). Calculation of the IAA amounts was performed using the CHEMSTATION software (Agilent).

IAA polar transport

Auxin transport assays were performed using with 5-day-old seedlings grown from seeds of a kanamycin-resistant plant so that a 1:2 ratio of wild-type:heterozygous seedlings resulted. To test transport inhibition by NPA, seedlings were transferred to agar plates containing 15 lM NPA 18 h prior to application of radioactive auxin. For measurement of non-inhibited transport, seedlings were transferred to NPA-free plates. The roots were 1.5–2 cm long, and the assays were performed as described by Lewis and Muday (2009), using agar cylinders as a source of radioactive 14C-IAA (Biotrend, http://www.biotrend.com). The final IAA concentration in the agar cylinder was 9 lM, corresponding to 0.5 lCi ml)1. Starting 1 mm away from the source of radioactive auxin, two 5 mm long pieces were cut, and the residual root was used as the third part (see Figure 7). The activity was measured after incubating the samples overnight at 4C in scintillation fluid. Seedlings were PCR-genotyped using the hypocotyl and cotyledons. Nucleic acid analysis For quantitative RT-PCR, 4 lg of total RNA was prepared using a NucleoSpin——————————— RNA plant kit according to the manufacturer's instructions (Macherey & Nagel, http://www.mn-net.com), and transcribed to first-strand cDNA using a RevertAidTM H Minus first-strand cDNA synthesis kit (Fermentas, http://www.fermentas.com). Primers were selected using PRIMER 3 software (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi) and NETPRIMER

software (http://www.premierbiosoft.com/netprimer/netprlaunch/netprlaunch.html), and checked for primer efficiency and agains primer dimer formation. The primers used were shown in table 1.

Table 1. List of Primer

Primer	Forward (5'-3')	Reverse (5'-3')
18S rRNA	GGCTCGAAGACGATCAGATACC	TCGGCATCGTTTATGGTT
ABP1	ACGAGAAAATCATACCAATTCGGACTAACC	GTATCTACGTAGTGTCACAAAACCTCAAC
IAA2	GGTTGGCCACCAGTGAGATC	AGCTCCGTCCATACTCACTTTCA
IAA11	CCTCCCTTCCCTCACAATCA	AACCGCCTTCCATTTTCGA
IAA12	CGTTGGGTCTAAACGCTCTG	TTCCGCTCTTGCTGCCTTCA
IAA13	CACGAAATCAAGAACCAAACGA	CACCGTAACGTCGAAAAGAGATC
IAA14	CCTTCTAAGCCTCCTGCTAAAGCAC	CCATCCATGGAAACCTTCAC
IAA19	GGTGACAACTGCGAATACGTTACC	CCCGGTAGCATCCGATCTTTTCA
IAA20	CAATATTTCAACGGTGGCTATGG	GCCACATATTCCGCATCCTCTA
GH3.5	AGCCCTAACGAGACCATCCT	AAGCCATGGATGGTATGAGC
SAUR9	GACGTGCCAAAAGGTCACTT	AGTGAGACCCATCTCGTGCT
SAUR15	ATGGCTTTTTTGAGGAGTTTCTTGGG	TCATTGTATCTGAGATGTGACTGTG
SAUR23	ATGGCTTTGGTGAGAAGTCTATTGGT	TCAATGGAGCCGAGAAGTCACATTGA

Quantitative PCR reactions were performed using 1 ll of sixfold diluted cDNA, 200 nM primers and 0.2· Power SYBR Green PCR master mix (Invitrogen, http://www.invitrogen.com/) in a StepOnePlusTM system (Applied Biosystems, http://www.appliedbiosystems.com/). For each pair of primers, the threshold value and PCR efficiency value were determined using cDNA diluted tenfold each time in five dilution steps. For all primer pairs, including the internal standard gene, 18S rRNA, the PCR efficiency was >99%. The specificity of PCR amplification was examined by monitoring the presence of a single peak in the melting curves for quantitative PCR. Amplicons were checked for fragment length on 4% agarose gels. For each determination, two to three biological repeats and three technical replicates for each determination were performed for the subsequent PCR reaction. Relative expression was calculated according to the ΔΔCt method using the equation:

relative expression = 2)[ΔCtsample) - ΔCtcontrol], where ΔCt = Ct(sample gene)); Ct(reference gene) and Ct refers to the threshold cycle determined for each gene in the early exponential amplification phase (Livak and Schmittgen, 2001). The expression level for the control treatment was set as 1-fold. For statistical analysis, REST 2008 software (Pfaffl et al., 2002) was used.

RESULTS

Morphological differences and physiological responses in abp1/ABP1 mutants

We grew seeds from heterozygous abp1/ABP1 plants on kanamycin-containing agar plates under a 16 h/8 h light/ dark cycle to eliminate wild-type plants (Ws background), before transferring resistant seedlings to soil. Resistant plants appeared to be smaller than wild-type plants (data not shown). This observation prompted us to investigate phenotypic properties of the abp1/ABP1 plants. PCR genotyping with primers against the insertion allele of ABP1 (Chen et al., 2001b) confirmed that resistant plants were heterozygous for abp1. Furthermore, viable seeds from abp1/ABP1 plants segregated 2:1 into resistant and wild- type seedlings on kanamycin-containing plates. Siliques of abp1/ABP1 contained approximately 25% non-viable white seeds, as described by Chen et al. (2001b).

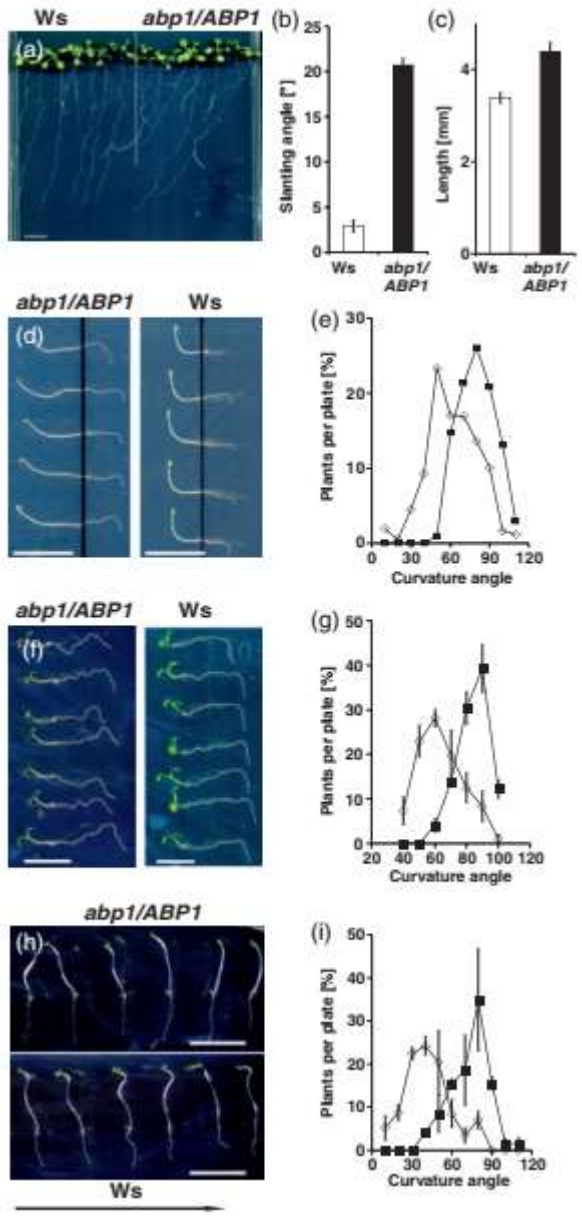


Fig. 1. Defects in gravitropic responses in shoot and roots of abp1/ABP1 mutants (a-g); Defects in phototropic responses of abl1/ABP1 shoots

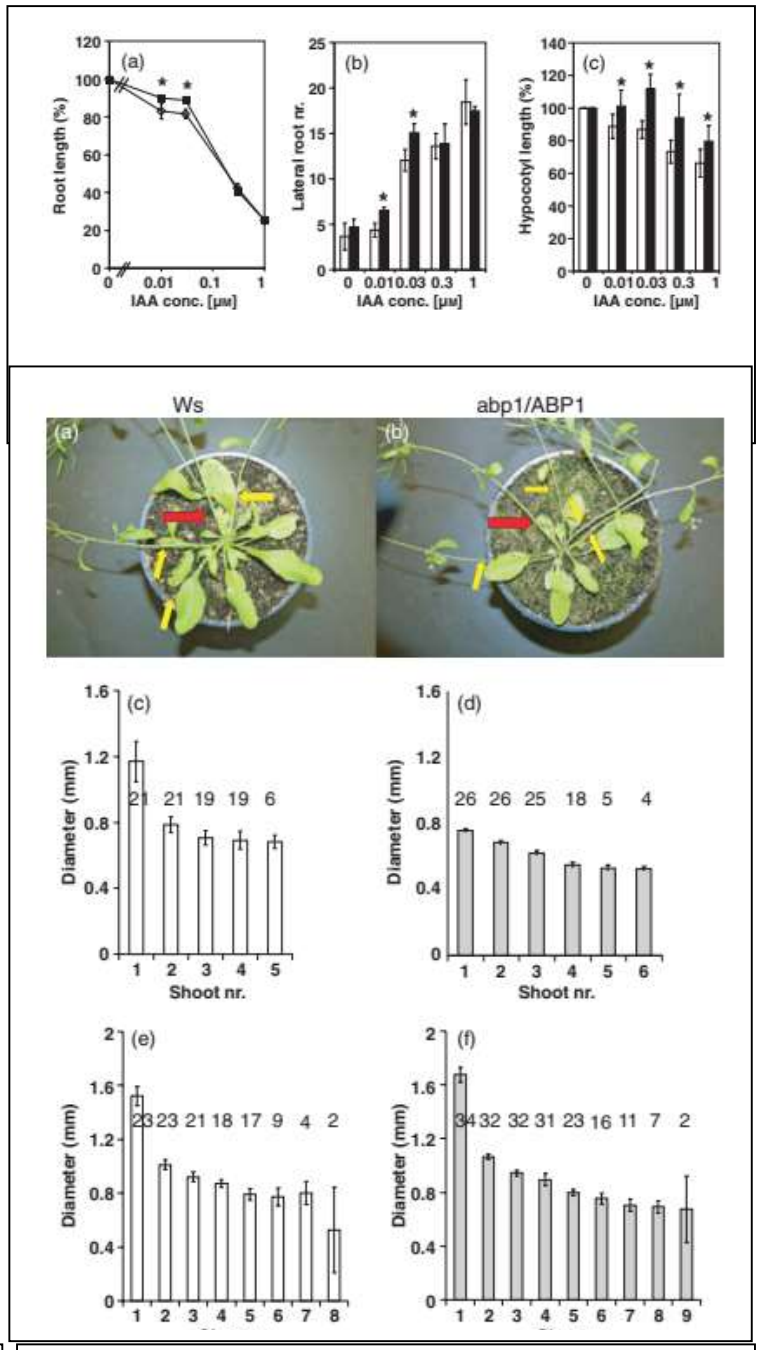


Fig. 2. Impairing responses to auxin application showed in roots and hypocotyls lengths in abp1/ABP1 mutants.

Fig. 3. More shoots in abp1/ABP1 mutants in comparison to WT, indicated a non-apical dominance in abp1/ABP1mutants

When we grew seeds from an abp1/ABP1 plant on kanamycin-free upright agar plates, we observed two seedling phenotypes: seedlings with roots growing down- wards, with only a small slanting angle, and seedlings with a strong slanting angle and roots that grew in a wavy pattern. Seedlings with a strong slanting angle were transferred to new agar plates after 4 days and grown side by side with wild-type seedlings treated the same way (Figure 1a,b). After 7 days, the selected mutant seedlings had a greater slanting angle (Figure 1a,b) and longer hypocotyls (Fig-ure 1c) compared to wild-type seedlings. The greater slanting angle suggested that heterozygous roots might be agravitropic. Therefore, we tested hypocotyls of 3-day-old dark-grown seedlings and roots of 14-day-light-grown seed- lings for defects in gravitropism by tilting the agar plates through 90° (Figure 1c–f). No pre-selection on kanamycin- free plates was performed prior to the experiment in order not to disturb or wound the seedlings as a result of transfer to a second medium. Seeds from wild-type and abp1/ABP1 plants were tested as separate populations. The distribution of bending angles of wild-type plants had a single peak centred at approximately 80° for hypocotyl gravitropism and approximately 90° for root gravitropism after 24 h. The population segregating for abp1 showed a peak at an angle of 50° and a smaller peak at 70–80° for hypocotyls, consistent with a segregation ratio of 2:1 for heterozygous versus wild-type plants (Figure 1d,e). Similar results were obtained for the gravitropic response of roots. While wild- type root bending angles peaked at 90°, the segregating population showed a peak at 60°, with a shoulder at 90° (Figure 1f,g). We then tested hypocotyl phototropism in dark-grown seedlings in a segregating population by application of 10 µmol m-2 s-1 of lateral blue light for 10 h. We again found a strong and uniform response in wild-type seedlings, with a peak of bending angles at approximately 80°, while a major response angle at 40° and a minor peak at 80° was observed in abp1/ABP1 progeny seedlings, reflecting the 2:1 segregation of this population (Figure 1h,i). The abp1 mutation therefore results in defects of gravitropism and phototropism, both developmental processes that are mainly controlled by auxin.

We tested auxin sensitivity in heterozygous and wild-type seedlings placed side by side on upright agar plates containing increasing auxin concentrations. Only small differences between wild-type and mutant in the length of the main root, lateral root formation and lateral root density were found in response to auxin (Figure 2a–c). These small differences were reproducible and may indicate a slight insensitivity of root and hypocotyl growth to auxin in the mutant.

Impairing of gene regulation in abp1/ABP1 mutants

Differences in phenotypes caused by altered auxin-related functions result from differential gene regulation. To investigate transcription of early auxin-regulated genes in response to auxin application, wild-type seedlings were grown in half-strength liquid MS medium and abp1/ABP1 progeny seeds were grown in medium containing kana- mycin. After 14 days, resistant seedlings were selected, and grown for 5 more days in kanamycin-free half-strength liquid MS medium, followed by auxin treatment. Wild-type seedlings were processed identically, omitting kanamycin throughout the selection procedure. At 30 min after treat- ment with 0.1 µM IAA, none of the seven IAA genes tested was up-regulated in abp1/ABP1 seedlings, but in the wild- type, IAA19 and IAA20 were more than tenfold up-regulated and IAA11, IAA13 and IAA14 were approximately two- to threefold up-regulated (Figure 5a). When seedlings were treated with 1 µM IAA, 11 of the 12 genes tested showed up-regulation in wild-type seedlings (Figure 5b), while five genes (IAA2, IAA14, SAUR9, SAUR15 and SAUR23) were not up-regulated at all in heterozygous seedlings and six were up-regulated, but to a lesser extent than in wild-type. IAA12 was down-regulated in wild type (Braun et al., 2008) but not in abp1/ABP1 seedlings. Only IAA11

and IAA19 expression was up-regulated to a similar level in heterozygous and wild-type seedlings in 1 µM IAA. Transcriptional stimulation was again generally higher after treatment with 10 µM IAA, and the expression levels for five of the 12 genes tested (IAA11, IAA13, IAA14, SAUR15, ABP1) were similar in heterozygous and wild-type seedlings, while the other genes were transcribed at lower levels in abp1/ABP1 than in wild-type seedlings (Figure 5c). IAA12 was slightly down-regulated by auxin in wild-type seedlings and weakly up-regulated in the mutant seedlings. In conclusion, transcriptional regulation of early auxin-response genes in heterozygous seedlings was less auxin-sensitive than in wild-type seedlings. When we compared expression of all tested genes in wild-type and mutant seedlings without auxin treatment, we found near-identical values for each, with differences of <4%. Surprisingly, ABP1 was itself an early auxin-regulated gene (Figure 5b,c). As for other auxin-inducible genes, ABP1 was up-regulated to a lesser extent by auxin in heterozygous mutant seedlings compared with the wild-type

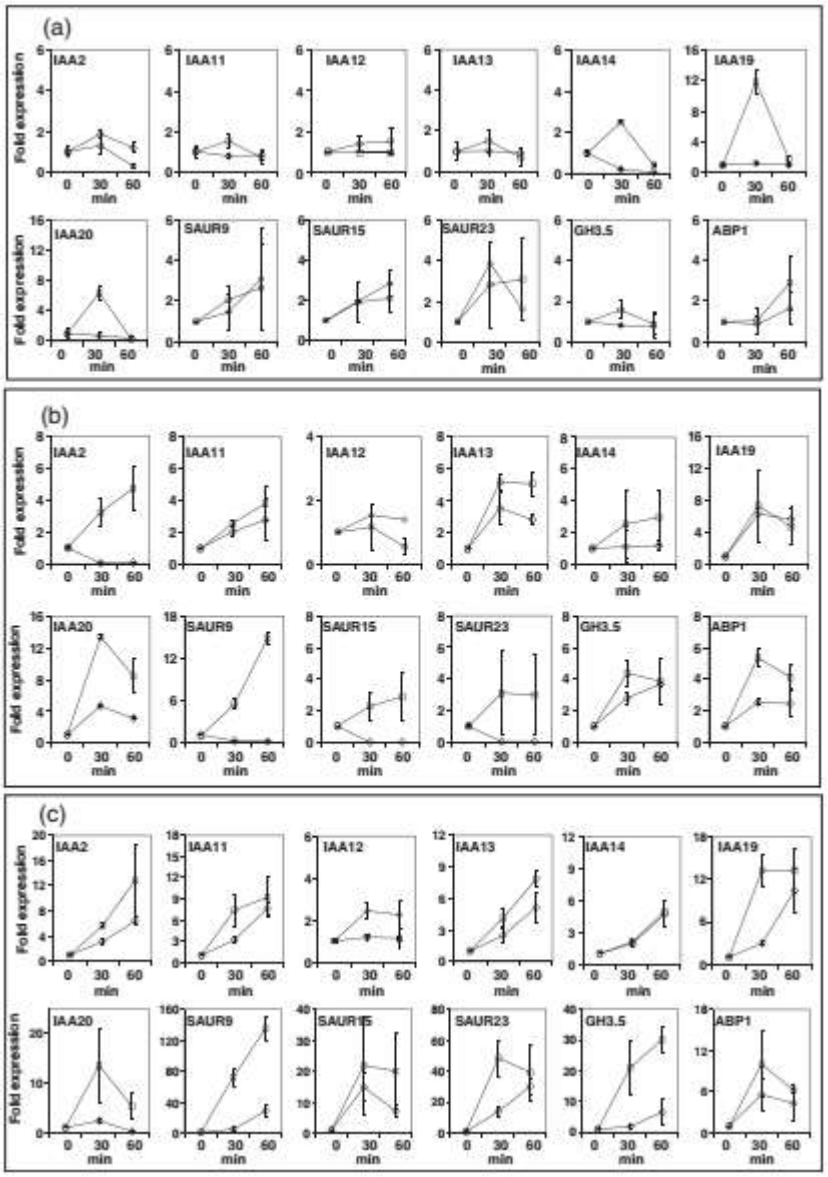


Fig. 5. Regulation of early auxin-regulated genes and ABP1 in light-grown wild-type (Ws) and abp1/ABP1 seedlings.

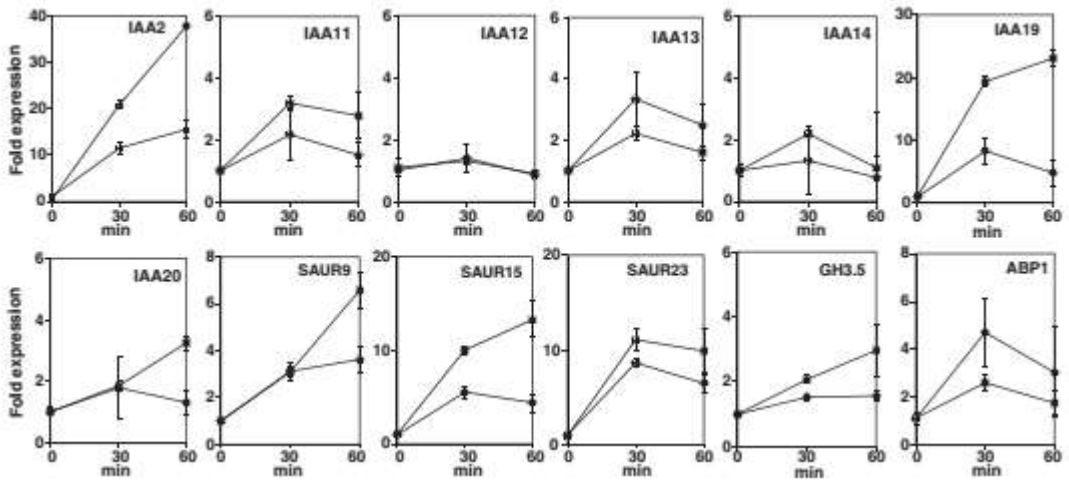


Figure 6. Regulation of early auxin-regulated genes and ABP1 in light-grown wild-type (Col) and eir1 seedlings.

DISCUSSION

Heterozygous abp1/ABP1 mutants exhibit morphological alterations Heterozygous abp1/ABP1 plants were previously described as having a similar phenotype to the wild-type (Chen et al., 2001b). Because the homozygous abp1/ABP1 mutant is embryo-lethal, only experiments with seed mixtures of 2:1 heterozygous:wild-type seeds are possible, and this may obscure investigations of the morphological phenotype. However, we found that heterozygous seed- lings have a waving and slanting root phenotype. Plants with similar phenotypes are often affected in auxin-related processes such as gravity perception or auxin physiology- related processes, or, alternatively, have

defects in microtubule-associated proteins (Sedbrook and Kaloriti, 2008). The abp1/ABP1 mutant had a defect in the gravi- tropic response of the root, which probably caused the root slanting.

The heterozygous mutants also had longer hypocotyls, which could be related to defective auxin or light signaling (Gray et al., 1998; Ljung et al., 2001; De Grauwe et al., 2005). Additionally, their phototropic response was impaired. In these respects, the abp1/ABP1 seedlings resemble mutants that are defective in phototropin-triggered phototropism. phototropin1 mutants exhibit long hypoctyls and defective phototropism (Chen et al., 2008), hence an association between the long-hypocotyl phenotype of abp1/ABP1 mutants and their phototropism defect seems possible.

In addition to altered gravitropism and phototropism and hypocotyl length, apical dominance was decreased in heterozygous plants (Figure 3), resulting in a semi-dwarf stature under long-day conditions. However, under short- day conditions, the size of the adult mutant plants was not different from that of adult wild-type plants, except for a slightly lower number of leaves in the early-flowering heterozygous plants, and the decrease in apical dominance was subtle (Figures 2 and 3). As the major contributor to apical dominance is auxin transport (Ongaro and Leyser, 2008), it is likely that the loss of apical dominance may be explained as a defect related to auxin physiology in the heterozygous abp1/ABP1 mutant.

Heterozygous abp1/ABP1 mutants exhibit defects in gravitropism and phototropism

The physiological phenotype of heterozygous abp1/ABP1 plants comprises defects in root and hypocotyl gravitro- pism, hypocotyl phototropism, polar auxin transport and an early-flowering phenotype. The common denominator for gravitropism and phototropism is regulation of polar auxin transport by PIN proteins (Petrásek et al., 2006), and mutants in which both gravitropism and phototropism are defective are comparatively few. The efflux transporters PIN2 and PIN3 have been identified on the basis of the properties of knockout or other mutants as contributing to both gravitropism and phototropism (Müller et al., 1998; Friml et al., 2002). Furthermore, two auxin signaling mutants, namely arf7 and iaa19, have been shown to be both agravitropic and aphototropic (Liscum and Reed, 2002). ARF7 and IAA19 are a transcription factor and a transcriptional co-factor, respectively, and the genes regulated by them, or a subset of these genes, must have a critical function in growth in tropisms. All other gravitropic or photo-tropic mutants are mutants in either gravitropism or phototropism alone, and their potential functional links to ABP1 are therefore weaker than proteins with functions in both gravitropism and phototropism. Our data show slower basipetal auxin transport in abp1/ABP mutant roots, but acropetal transport was not significantly altered. PIN2- mediated basipetal auxin transport is required for root gravitropism (Wisniewska et al., 2006; Abas et al., 2006; Michniewicz et al., 2007). We therefore suggest that ABP1 acts through the activity changes of PIN proteins on gravitropism and auxin-related functions observed in the heterozygous plants.

Mutation in ABP1 gene strongly influence auxin function, including transcription of early auxin-regulated genes

The most surprising aspect of our findings was that the transcriptional regulation of all early auxin-induced genes tested was lower or slower in the heterozygous abp1/ABP1 plants than in wild-type seedlings (Figure 5). Summarizing results from treatments with three IAA concentrations, the heterozygous plants can be considered as a partly auxin- insensitive mutant with respect to short-term gene regulation of early auxin genes. The changes in phototropism after 10 h, gravitropism after 24 h and basipetal polar auxin transport in the root after 18 h are relatively immediate responses, and reduced sensitivity was observed in all these tests. By contrast, in the 12-day growth assay in an auxin concentration series, we observed only a slight difference in auxin sensitivity of mutant seedlings compared with wild-type. Thus, ABP1 function is better revealed in studies of short-term regulation of auxin responses rather than tests over a longer period of time, in which a signaling network tends to dampen defects.

Our data on early auxin-induced gene regulation show that all tested IAA genes were mis-regulated in the heterozygous abp1/ABP1 mutant, including IAA19, the SAUR genes, GH3.2 and ABP1 (Figure 5). In an attempt to elucidate the mechanism behind this mis-regulation, we investigated the regulation of these test genes in the PIN2 mutant eir1 (Chen et al., 1998; Luschnig et al., 1998; Müller et al., 1998). Mis-regulation of PIN2 could be the reason for the observed defect in root basipetal auxin transport (Abas et al., 2006; Wisniewska et al., 2006; Sukumar et al., 2009). Indeed, eight of 12 genes tested were up-regulated to a lower extent in the eir1 background compared with the wild- type, one gene was not differentially expressed, and three were up-regulated by auxin to a greater extent (Figure 6). A defect in PIN2 could become manifest by defects in regulation of the same set of genes, suggesting that ABP1 and PIN2 occur in largely overlapping regulatory pathways. Our suggestion is that ABP1 and PIN proteins cooperate in a tight regulatory circuit (Figure 7). The differences in the „signature“ of regulation of early auxin

genes between abp1/ ABP1 and eir1 could be explained by participation of additional proteins that regulate cellular auxin concentration. The most likely candidates are other PIN proteins and AUX1 or LAX proteins. Alternatively, mis-expresssion of early auxin genes could be due to a direct effect of ABP1 on TIR1-dependent IAA ubiquitination. However, there is currently no evidence for this second explanation. The mechanisms are not mutually exclusive (Figure 7). Common to all aspects of the abp1/ABP1 phenotype is that they may be explained by changes in polar auxin transport regulation and local auxin concentration.

A possible crosstalk between ABP1 and TIR1 as receptor regulating the early auxin-regulated genes

Although auxin binding to ABP1 does undoubtedly occur (Napier et al., 2002), it has gained little acceptance as an auxin receptor. A reason for this might be the lack of knowledge as to how exactly a potential ABP1-induced signal pathway is connected to the cytosol-based regulatory mechanisms of signal transduction. Although a number of such reactions have been shown, the receptor(s) was not unequivocally identified (Scherer and Andre, 1989; Paul et al., 1998; Tao et al., 2002; Shishova et al., 2007; Lanteri et al., 2008). Furthermore, the postulated docking protein for ABP1 (Kla¨ mbt, 1990) needs to be identified for full understanding of ABP1 action. Interestingly, gene dosage effects, such as the haplo-insufficiency for ABP1 reported here, often relate to gene products that interact with other proteins strictly stoichiometrically (Veitia et al., 2008), and are found for various human receptors (Fisher and Scambler, 1994). If ABP1 does indeed require a docking protein for its function, the observed haplo-insufficiency in the heterozygous plants could be due to stoichiometric imbalance.

Direct regulation of early auxin-regulated genes has been shown convincingly (Mockaitis and Estelle, 2008), but can the same genes regulated by TIR1-dependent ubiquitination of IAA proteins also be regulated by a different receptor? Our results show that ABP1 is required for the regulation of early auxin-regulated genes. However, further experiments are required in order to determine whether ABP1 acts independently of the function of TIR1 and its homologs. It has been speculated that the regulation of polar auxin transport by auxin might be independent of SCFTIR1/AFB signalling (Paciorek et al., 2005), suggesting that an auxin receptor other than TIR1 and its homologs is required. We show here that not only is the transcription of early- regulated auxin genes altered in the heterozygous abp1/ ABP1 mutant, but also tropic responses that are commonly associated with regulation of polar auxin transport.

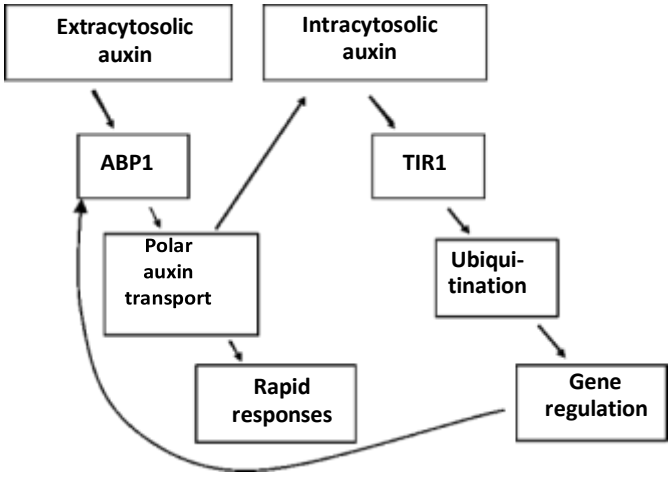


Figure 8. A hypothetical crosstalk signal transduction involving ABP1 and TIR1 as auxin receptors.

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