J Plant Growth Regul (2011) 30:416–424
DOI 10.1007/s00344-011-9203-2

Expression and Subcellular Localization of *Arabidopsis thaliana* Auxin-Binding Protein 1 (ABP1)

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Received: 19 November 2010 / Accepted: 9 February 2011 / Published online: 19 April 2011

Abstract Expression of *Arabidopsis thaliana ABP1* (*AUXIN-BINDING PROTEIN 1*) was studied using a promoter:GUS approach. Two promoter regions were analyzed. The 1585-bp promoter region upstream of the translation start site (P_{ABP1}) showed different activity compared to the promoter region that included, in addition, the first two introns and three exons of the transcribed *ABP1* sequence ($P_{ABP1i1,2}$), indicating that *cis* elements were present downstream of the start codon. $P_{ABP1i1,2}$-driven β-glucuronidase activity was highest in growing leaves, in the root meristem, in vascular tissues, and in hydathodes. *ABP1* promoter activities overlapped largely but not completely with that of DR5, which is a marker for the ARF-AuxRE-dependent auxin response. Subcellular ABP1 localization was studied using a constitutively overexpressed EGFP-ABP1 fusion protein. Results confirmed predominant localization to the endoplasmic reticulum as was concluded previously.

Keywords Auxin · Auxin-binding protein 1 · ABP1:GUS · DR5:GUS · Subcellular localization

Introduction

Auxin regulates plant development and growth. Its polar transport *in planta* is mediated by influx carriers of the AUX1/LAX (AUXIN-RESISTANT1/LIKE AUX1) family and by efflux carriers of the PGP (P-glycoproteins of the ABCB transporter family) or PIN (PIN-FORMED) families. Polar auxin transport was shown to be crucial for cell patterning, organ development, and tropisms (Friml 2007; Petrásek and Friml 2009; Ugartechea-Chirino and others 2010). PIN activity is regulated by auxin-mediated cycling between endosomes and the plasma membrane (Geldner and others 2001; Friml and others 2002; Paciorek and others 2005; Dhonukshe and others 2007). Auxin is perceived by two types of receptor proteins. TIR1 (TRANSPORT INHIBITOR RESPONSE 1) and the related proteins AFB1–5 (AUXIN SIGNALING F BOX PROTEIN 1–5) are part of E3 ligase complexes of the SCF type, which regulate degradation of proteins through the proteasome pathway (Dharmasiri and others 2005; Kepinski and Leyser 2005; Tan and others 2007). SCF^{TIR1} promotes degradation of transcriptional repressors of the Aux/IAA family, which results in auxin-induced gene transcription. Aside from the TIR1/AFB proteins, AUXIN-BINDING PROTEIN 1 (ABP1) was identified as an auxin receptor.

ABP1 was first described as a protein that binds auxin and was proposed to act as an auxin receptor (Hertel and others 1972; Löbler and Klämbt 1985). ABP1 binds natural and synthetic auxins at physiological concentrations (Jones and Venis 1989). Transient changes of the membrane potential of tobacco protoplasts (Barbier-Brygoo and others 1989, 1991) and protoplast swelling (Steffens and others 2001; Yamagami and others 2004; Dahlke and others 2009) are sensitive to immunological tools and synthetic peptides related to ABP1, strengthening the idea that ABP1 acts as an auxin receptor (Venis 1995). Knock out of the single *ABP1* gene in *Arabidopsis thaliana* is embryo lethal (Chen and others 2001a), whereas modulation of *ABP1* expression in *Arabidopsis* and in tobacco resulted in altered shoot growth and

Electronic supplementary material The online version of this article (doi:10.1007/s00344-011-9203-2) contains supplementary material, which is available to authorized users.

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development (Braun and others 2008). In *Arabidopsis*, ABP1 was shown to alter root growth in an auxin-dependent manner by modulating cell proliferation and cell elongation (Tromas and others 2009). ABP1 was further shown to regulate clathrin-mediated endocytosis and thereby auxin transport via PIN proteins (Robert and others 2010).

The ABP1 preprotein contains a signal peptide and a conserved KDEL sequence, indicative of localization in the endoplasmic reticulum (ER). ER localization of ABP1 in maize was concluded early based on subcellular fractionation (Ray 1977). A minor fraction of ABP1 appeared to escape ER retention and to pass on to the apoplast, as deduced from immunolocalization studies (Jones and Herman 1993; Diekmann and others 1995; Henderson and others 1997). Whether this is due to active export or results from failure of the retention mechanism is not known (Henderson and others 1997).

We performed subcellular localization studies of *Arabidopsis* ABP1 using an EGFP-ABP1 fusion protein, which confirmed predominant localization of ABP1 to the ER. Furthermore, *ABP1* expression was studied using two promoter constructs and was compared to the expression pattern of DR5:GUS.

Materials and Methods

Plant Growth Conditions

Wild-type *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia seeds were originally obtained from GABI-Kat (Max-Planck-Institut, Cologne, Germany). For experiments in sterile conditions, seeds were surface sterilized in 2% (v/v) sodium hypochlorite for 20 min, washed five times with water, and placed in 50 ml of 0.5 × Murashige and Skoog medium at pH 5.8 (Murashige and Skoog 1962; DUCHEFA Biochemie, Haarlem, The Netherlands) containing 0.38% (w/v) Gelrite (DUCHEFA Biochemie, Haarlem, The Netherlands) and 1.5% (w/v) sucrose (Carl Roth, Karlsruhe, Germany) in square plates (Greiner Bio-One, Frickenhausen, Germany). Seeds were stratified for 3 days at 4°C. Etiolated seedlings were kept at 22°C. Plants were raised in a growth chamber under long-day conditions (16 h light, 8 h dark, 22°C). DR5:GUS seeds were kindly provided by Tim Ulmasov (Missouri University, Columbia, MO, USA).

Cloning and Transformation

For expression analysis of *ABP1* by promoter:GUS, two promoter fragments were cloned from the *ABP1* gene (At4g02980). For the first promoter construct, 1585 bp upstream of ATG were amplified by PCR using oligonucleotides FW_PG and RV_PG-A1 (For primers see Supplementary Table 1). For the second construct, the same fragment was amplified and, in addition, the N-terminal coding region of *ABP1*, including introns 1 and 2, was amplified with oligonucleotides FW_PG-B2 and RV_PG-B2. The 5′ amplification product was joined to the transcribed region by PCR using the overhangs introduced. The full-length sequence was further amplified using oligonucleotides FW_PG and RV_PG_B2. Both promoter fragments were cloned into pENTR-D-TOPO (Life Technologies, Carlsbad, CA, USA) by directional topoisomerase-mediated cloning and transferred to pBGWFS7 (Karimi and others 2002) by an LR reaction to create P_{ABP1}:GUS, with the 5′ promoter fragment and $P_{ABP1i1,2}$:GUS with the same 5′ region and transcribed sequences (Fig. 1a, b).

To create an EGFP-ABP1 overexpression construct, the signal peptide of *ABP1* was placed in front of the EGFP coding sequence (Yang and others 1996). An additional c-myc tag was introduced behind the signal peptide. The EGFP coding sequence was linked to the ABP1 coding sequence lacking the signal sequence by a flexible linker sequence (GSAGSAAGSG; Waldo and others 1999) to minimize interference of EGFP with ABP1 and vice versa (Fig. 1c). The signal peptide of ABP1 was amplified by PCR using oligonucleotides FW_ABP1_N and RV_ABP1_SPmyc. The c-myc tag was added by amplifying the product of the previous PCR with primers FW_ABP1_N and

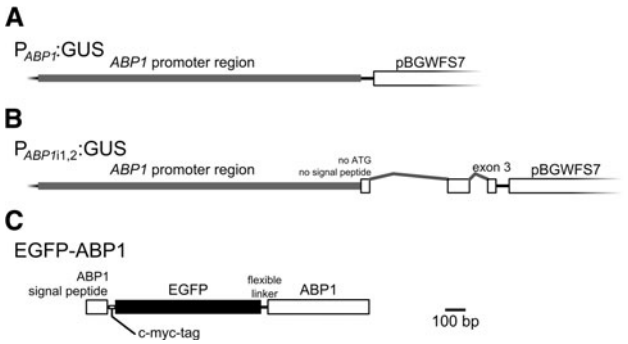

Fig. 1 Promoter:GUS and protein fusion constructs. **a**, **b** Two promoter:GUS constructs were generated to study expression of *ABP1*. The *β*-glucuronidase coding sequence is part of the pBGWFS7 vector (Karimi and others 2002). **a** P_{ABP1}:GUS contains 1585 bp upstream of the start codon of *ABP1* cloned in front of the *β*-glucuronidase reporter. **b** $P_{ABP1i1,2}$:GUS contains 1585 bp upstream of the start codon and the first three exons and two introns of the *ABP1* gene cloned in front of the *β*-glucuronidase reporter. The start codon and signal peptide were deleted in both constructs to prevent premature translation initiation and ER localization of the GUS protein. **c** The EGFP-ABP1 construct was generated to allow for subcellular localization of ABP1. The native ABP1 signal peptide was cloned 5′ of the EGFP coding sequence, and this, in turn, was fused to the sequence encoding the mature ABP1 protein. A flexible linker was introduced in frame between EGFP and ABP1 to prevent disruption of protein functions. Furthermore, a c-myc tag was introduced N-terminal to EGFP

RV_ABP1_SPmyc2. EGFP was amplified from the vector pJCGLOX (Joubes and others 2004) with FW_mycEGFP and RV_EGFP. The product of this PCR and the ABP1 signal peptide/c-myc-tag sequence were fused by overlap PCR using primers FW_ABP1_N and RV_EGFP. The remaining part of the ABP1 coding sequence was amplified with oligonucleotides FW_ABP1_Eco32I and RV_ABP1_C and further extended at the 5′ end using FW_ABP1_E9C, FW_ABP1_E9D, FW_ABP1_E10, and FW_ABP1_E11, each time combined with oligonucleotide RV_ABP1_C. For each PCR extension, the product of the previous amplification was used as a template. To fuse the resulting fragment with the ABP1-signal-peptide-EGFP sequence, another overlap PCR with oligonucleotides FW_ABP1_N and RV_ABP1_C was performed. The resultant sequence was cloned into pENTR-D-TOPO, sequenced, and then transferred into pB7WG2 (Karimi and others 2002), which drives expression through the 35S promoter.

For *Arabidopsis* transformation, a modified floral dip method (Logemann and others 2006) and *Agrobacterium tumefaciens* were used. After selection, 11 transgenic plants were recovered for the P_{ABP1}:GUS transformants and 24 transgenic plants for the $P_{ABP1i1,2}$:GUS transformants. For EGFP-ABP1, 17 lines were found to be resistant to glufosinate and were further analyzed for protein expression using Western blot and fluorescent microscopy.

Immunoblot and Deglycosylation Assay

Expression of EGFP-ABP1 was verified by Western blot analysis. One rosette leaf each from adult plants was harvested from the lines analyzed and ground in protein-loading buffer consisting of 10% (w/v) glycerol, 5% (v/v) β-mercapto ethanol, 1% (w/v) sodium dodecyl sulfate (SDS), 62.5 mmol l^{-1} Tris-HCl (pH 6.8), and 0.5% (w/v) bromophenol blue (Carl Roth, Karlsruhe, Germany). Crude protein extract was incubated at 98°C for 5 min and centrifuged to dispose of cell debris. Ten microliters of the supernatant were separated on a denaturing 12.5% SDS polyacrylamide gel and blotted onto a polyvinylidene difluoride membrane (Amersham Hypond-P, GE Healthcare, Munich, Germany). The membrane was washed in Tris-buffered saline with Tween-20 and bovine serum albumin (TBSTB) containing 20 mmol l^{-1} Tris-HCl, 137 mmol l^{-1} sodium chloride, 0.1% (v/v) Tween-20, 0.03% (w/v) bovine serum albumin (pH 7.6), and was blocked for 1 h in 5% (w/v) low-fat powdered milk in TBSTB. The blot was then incubated for 1 h at room temperature with the primary monoclonal anti-c-myc antibody 9E10 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), diluted 1:20,000 in TBSTB, and washed six times in TBSTB for a total of 35 min at room temperature. The blot was then incubated with a secondary goat-anti mouse antibody linked to horseradish peroxidase (Santa Cruz Biotechnology) diluted 1:40,000 in TBSTB, and washed as indicated above. Horseradish peroxidase activity was detected by chemiluminescence (Amersham ECL Plus, GE Healthcare, Munich, Germany) and visualized on an X-ray film (Amersham Hyperfilm MP, GE Healthcare, Munich, Germany).

For the deglycosylation assay, two rosette leaves of 3-week-old plants were ground in liquid nitrogen and resuspended in protein isolation buffer with 20 mmol l^{-1} Tris-HCl, 100 mmol l^{-1} potassium chloride, 0.5% (v/v) *tert*-octylphenoxy poly(oxyethylene)ethanol (pH 7). Tissue and cell debris were pelleted by centrifugation and discarded. The protein concentration of the supernatant was measured by Bradford assay (Roti-Quant, Carl Roth, Karlsruhe, Germany). PNGase F treatment was performed according to the manufacturer's instructions (New England Biolabs, Ipswich, MA, USA).

Histochemical GUS Detection and Fluorescence Microscopy

Five independent, glufosinate-resistant P_{ABP1}:GUS and $P_{ABP1i1,2}$:GUS lines with strong GUS activity were analyzed for GUS expression. Individual plants showed comparable staining patterns. GUS staining was performed as previously reported (Vielle-Calzada and others 2000). Stained tissue was cleared in 60% (w/v) chloral hydrate (Yadegari and others 1994) and observed under bright-field illumination (Olympus BX41 microscope, Olympus, Hamburg, Germany). Five-day-old de-etiolated $P_{ABP1i1,2}$:GUS seedlings were stained, dehydrated in an ethanol series, and embedded in Technovit 7100 according to the manufacturer's instructions (Heraeus Kulzer GmbH, Wehrheim, Germany). Twenty-five-micron-thick sections were cut using an RM 2255 microtome (Leica Microsystems, Wetzlar, Germany) and analyzed using an Olympus BX41 microscope. For subcellular localization of the EGFP-ABP1 fusion protein, 5-day-old etiolated seedlings were mounted on a glass slide and observed using a confocal laser scanning microscope (Leica TCS SP, Leica Microsystems, Wetzlar, Germany). Four independent lines were analyzed and representative patterns were observed in a minimum of 20 plants each.

Binding Site Prediction

To identify putative transcription factor binding sites downstream of the transcription start site (TSS) of *ABP1*, −200 bp and +750 bp relative to the TSS of *ABP1* were submitted to AthaMap (Bülow and others 2009) using 20% restriction to highly conserved binding sites. The graphic

output was retrieved and visually revised for presentation. Factors assigned to the category "others," for example, TATA-binding protein, were omitted for clarity. ConSite (Sandelin and others 2004) binding site predictions were retrieved using the same sequence with a minimum specificity of ten bits and using only the four *Arabidopsis* transcription factors available (namely, AGAMOUS, AGAMOUS-LIKE 3, *Arabidopsis thaliana* HOMEOBOX 1 [ATHB1], and ATHB5). Binding sites predicted by ConSite were manually added into the AthaMap graphical output at the appropriate positions.

Results

The *ABP1* Promoter Extends into the Transcribed Region

To determine tissue-specific expression of *ABP1*, promoter:GUS studies were performed. To that end, two different constructs were generated and transformed into *Arabidopsis*. The P_{ABP1}:GUS construct contained 1585 bp upstream of the translation start codon (Fig. 1a). The $P_{ABP1i1,2}$:GUS construct contained the same upstream region and also included 664 bp of the transcribed region encompassing the first three exons with two introns from which the start codon and signal peptide were deleted (Fig. 1b). Expression patterns obtained with these two promoters were compared to expression of DR5:GUS, which is a marker for ARF-AuxRE-dependent auxin responses (Ulmasov and others 1997). GUS activity was analyzed in developing light-grown and etiolated seedlings and in leaves.

In 1-day-old etiolated or de-etiolated P_{ABP1}:GUS seedlings, no GUS staining was observed in the root (Fig. 2a, p). Staining was observed in seedlings starting 2 days post germination, mostly in the hypocotyl and cotyledons (Fig. 2d, g, j, m, s, v, y, bb). It became weaker as the seedling grew older such that 5-day-old seedlings had to be stained for more than 24 h, compared to about 18 h for 2-day-old seedlings, to observe GUS activity. Promoter activity was observed at the root-to-shoot transition of 2-day-old seedlings (Fig. 2d). Etiolated seedlings had a staining pattern similar to that of de-etiolated seedlings. In addition, etiolated seedlings displayed staining at the hypocotyl hook, mainly of the vascular tissue (Fig. 2s, y, bb).

In $P_{ABP1i1,2}$:GUS seedlings, staining was observed at the root tip 1 day post germination but was absent from the root cap. This staining pattern persisted as the seedlings grew older (Figs. 2b, e and 3e). Some promoter activity was observed at the root-to-shoot transition of 2–5-day-old seedlings (Fig. 3a–c). In roots, staining was further observed in the vascular tissue (Fig. 4b, d), including the

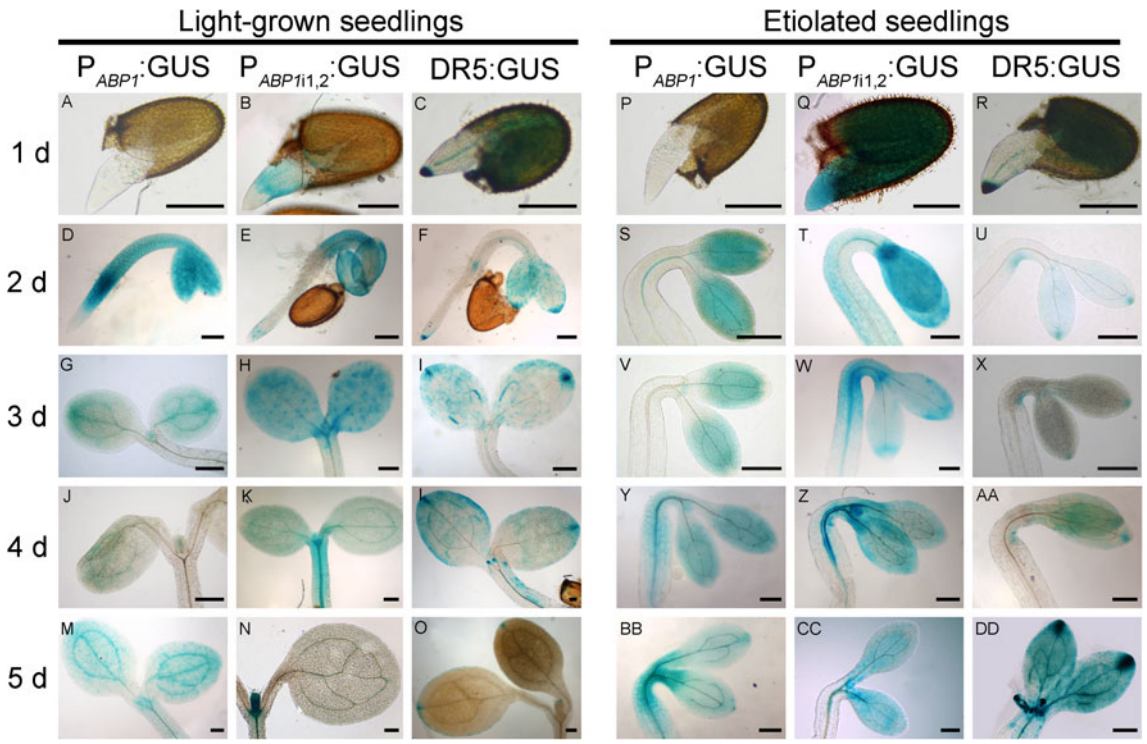

Fig. 2 Comparison between P_{ABP1}:GUS, $P_{ABP1i1,2}$:GUS, and DR5:GUS activities. **a–o** De-etiolated or **p–dd** etiolated P_{ABP1}:GUS, $P_{ABP1i1,2}$:GUS, and DR5:GUS seedlings were grown for 1–5 days and analyzed for β-glucuronidase activity. Scale bars = 200 μm

pericycle (Supplementary Fig. 1). Lateral root formation was accompanied by strong staining throughout the primordium (Figs. 3d and 4d). Older lateral roots revealed the same staining pattern seen in the main root (Fig. 2e).

The hypocotyl revealed $P_{ABP1i1,2}$:GUS activity in 2-day-old seedlings (Fig. 2e, t). This activity declined in older seedlings, with only the very apical part of the hypocotyl being stained after 5 days (Fig. 2n, cc). Entire cotyledons were stained (Fig. 2e, h, k, t, w, z, cc), occasionally with a patchy pattern (Fig. 2h), and strong staining was observed in the first true leaves (Fig. 2n, z, cc). The staining patterns did not vary greatly between etiolated and light-grown seedlings, indicating that $P_{ABP1i1,2}$:GUS expression was not light-dependent.

DR5:GUS activity was localized to known sites of maximal auxin concentration and responsiveness, such as the root tip (Figs. 2c, r and 4h), the tip of cotyledons (Fig. 2f, u), and the tip of young leaves (Fig. 2l). In etiolated seedlings, asymmetrical distribution of staining was observed at the hypocotyl hook (Fig. 2u, x, aa) that was not visible in light-grown seedlings (Fig. 2f, i, l). Analysis of GUS expressions in leaves of 3-week-old plants showed no detectable signal in P_{ABP1}:GUS lines (Fig. 5a, d). Leaves of $P_{ABP1i1,2}$:GUS (Fig. 5b, e) and DR5:GUS (Fig. 5c, f) displayed staining with highest GUS activity at leaf hydathodes.

A comparison between P_{ABP1}:GUS, $P_{ABP1i1,2}$:GUS, and DR5:GUS staining patterns revealed highly overlapping expression between $P_{ABP1i1,2}$:GUS and DR5:GUS in the vascular tissue, at the root tip, and in young leaves (Figs. 2 and 4). However, the expression pattern conferred by the expanded *ABP1* promoter sequence in $P_{ABP1i1,2}$ did not completely match the DR5-driven expression. The most remarkable difference was observed at the hypocotyl hook region (Fig. 4c, f). DR5:GUS expression was asymmetrical, with blue staining found only at the concave side, whereas

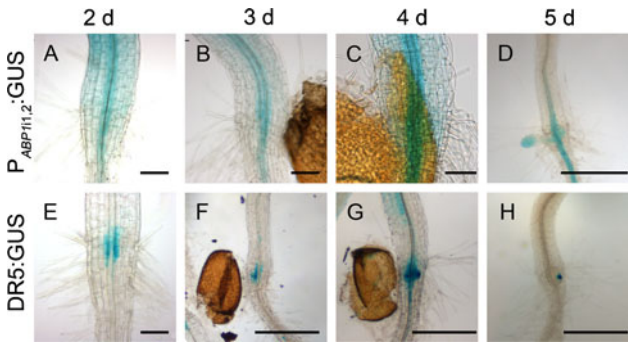

Fig. 3 Comparison between $P_{ABP1i1,2}$:GUS and DR5:GUS activities at the root-to-shoot transition. **a**–**c**, **e**, **f** GUS staining at the root-to-shoot transition zone. **d**, **g**, **h** Lateral roots and primordia are visible in roots of older seedlings. Scale bars = 500 μm (**d**, **f**–**h**) and 100 μm (**a**–**c**, **e**)

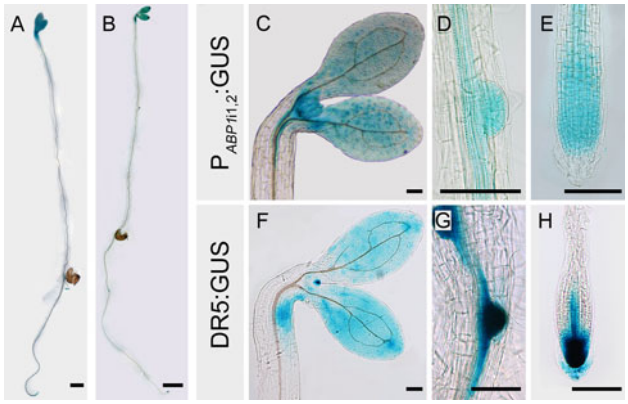

Fig. 4 $P_{ABP1i1,2}$:GUS and DR5:GUS activities in roots and shoots of etiolated 5-day-old seedlings. **a**, **c**, **d**, **e** $P_{ABP1i1,2}$:GUS seedlings. **b**, **f**, **g**, **h** DR5:GUS seedlings. *β*-Glucuronidase activities differ at the apical hook region, but are comparable in (**c**, **f**) cotyledons, developing leaves, (**d**, **g**) lateral root primordia, and (**e**, **h**) at the root tip. Scale bars = 500 μm (**a**, **b**) and 100 μm (**c**–**h**)

$P_{ABP1i1,2}$:GUS was localized to the vascular tissue. The P_{ABP1} promoter showed overlapping activity with $P_{ABP1i1,2}$ and DR5 in cotyledons and vascular tissue but was not active in the root tip and at leaf hydathodes.

As the promoter:GUS study revealed that expression of *ABP1* was in part controlled by promoter sequences that are located downstream of the translation start site, computational analyses were performed. ConSite (Sandelin and others 2004) and AthaMap (Bülow and others 2009) predicted several transcription factor binding sites within this sequence, for example, for MYB and AP2/ERF-type transcription factors (Supplementary Fig. 2). It is conceivable that additional, unpredicted binding sites exist within this region.

Protein Expression and Subcellular Localization of EGFP-ABP1

A fusion construct was generated for subcellular localization of the *Arabidopsis* ABP1 protein (Fig. 1c). For versatility, two tags, c-myc and EGFP, were introduced. To that end, the ABP1 signal peptide was fused in front of a c-myc tag, followed by the coding sequence of enhanced GFP protein (EGFP) and by the ABP1 coding region without the signal peptide. ABP1 has a C-terminal ER retention signal and the C-terminus was proposed to participate in auxin binding (Thiel and others 1993; Leblanc and others 1999; Steffens and others 2001). Therefore, the c-myc tag and the coding sequence of the fluorescent protein EGFP were fused N-terminal of the ABP1 coding region but C-terminal of the native ABP1 signal peptide to retain proper targeting of the fusion protein. EGFP-ABP1 was expressed under control of the 35S promoter. Proper expression of the fusion protein was analyzed in two ways.

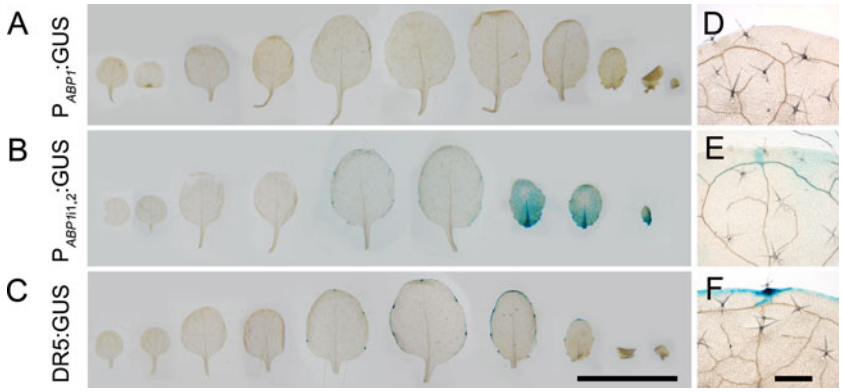

Fig. 5 P_{ABP1}:GUS, $P_{ABPi1,2}$:GUS, and DR5:GUS activities in leaves of 3-week-old plants. (**a**, **d**) P_{ABP1}:GUS, (**b**, **e**) $P_{ABPi1,2}$:GUS, and (**c**, **f**) DR5:GUS plants were grown in long-day conditions for 3 weeks. **a–c** Cotyledons and leaves were stained for β-glucuronidase activity and arranged according to age from left (oldest) to right (youngest). Scale bar = 10 mm. **d–f** Magnified views of the fourth leaf from each line. Hydathodes of (**e**) $P_{ABPi1,2}$:GUS and (**f**) DR5:GUS but not of (**d**) P_{ABP1}:GUS display staining. Scale bar = 0.5 mm

First, expression and size of the expressed protein were tested in lines 1–7 by Western blot using an anti-c-myc antibody (Fig. 6a). Expression of the fusion protein was detected in lines 3, 5, and 7. The size of the expressed protein was estimated at around 55 kDa. The expected size of the fusion protein is 51.4 kDa. Maize ABP1 was described as a glycosylated protein (Woo and others 2002). We therefore tested if the overexpressed fusion protein might be similarly modified. Crude protein extract from *Arabidopsis* leaves was digested with PNGase F to release N-linked carbohydrate moieties or was left untreated as a control (Fig. 6b). After PNGase F treatment, the size of the fusion protein shifted from 55 kDa to about 50 kDa, which approximates the expected size of the unmodified fusion protein. The observation that the fusion protein displayed the expected size and was modified by glycosylation, as was described for the native maize protein, indicated that it was properly synthesized and processed.

We next studied the subcellular localization of EGFP-ABP1 using fluorescence microscopy. Longitudinal sections from the root hair zone were analyzed by confocal laser scanning microscopy (Fig. 6c, e). A fluorescence signal was observed in a reticulate network pattern that is typical of the ER found in vacuolated cells (Fig. 6e). Fusiform bodies typical of ER-localized GFP fusion proteins (Hawes and others 2001) were also observed. EGFP-ABP1 fluorescence was found around the nucleus, which again is typical for ER-localized proteins (see Supplementary video). EGFP-ABP1 localization was also analyzed in the epidermis of cotyledons (Fig. 6d). An enlarged view of guard cells again showed a fluorescence signal in a reticulate network pattern (Fig. 6f). The results thus indicate that the fusion protein was properly processed and was predominantly localized to the ER.

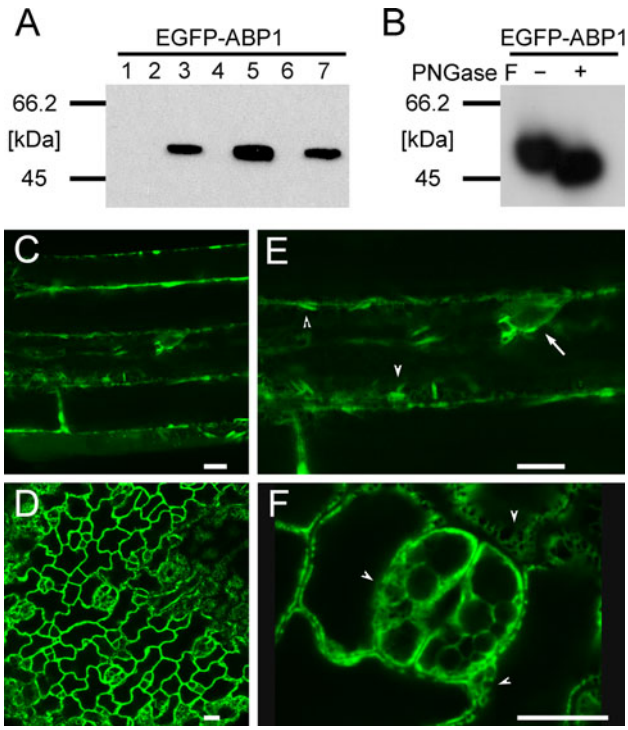

Fig. 6 Detection and subcellular localization of EGFP-ABP1 fusion protein. **a** Transgenic lines were analyzed for EGFP-ABP1 expression using immunoblotting. Crude protein extract was isolated from a rosette leaf of EGFP-ABP1 lines 1–7, separated on a SDS gel, and blotted. For EGFP-ABP1 detection, the primary anti-c-myc antibody 9E10, at a dilution of 1:20,000, and a secondary goat anti-mouse IgG antibody, at a dilution of 1:40,000, were used. **b** To test if the fusion protein was glycosylated, extracts were treated with or without PNGase F prior to Western analysis. **c–f** To study subcellular distribution of EGFP-ABP1, cells (**c**, **e**) in the root hair zone and (**d**, **f**) in cotyledons were analyzed by confocal laser scanning microscopy. Arrow points to a nucleus. Arrowheads point to the reticulate pattern and to fusiform bodies typical of ER-localized GFP expression. Scale bar = 10 μm

Discussion

Auxin was shown to regulate cell proliferation and cell growth through binding to its designated receptor ABP1 (David and others 2007; Braun and others 2008; Tromas and others 2009). It might be expected that the single *ABP1* gene of *Arabidopsis* is expressed in meristematic cells and growing tissues, and possibly in tissues with a potential for cell division and growth. To identify actual sites of *ABP1* activity, promoter:GUS expression studies were performed using two promoter fragments. The expression pattern obtained with the 1585-bp 5′ upstream promoter region of *ABP1* did not agree with data obtained from microarray studies (Genevestigator; Hruz and others 2008). Therefore, an extended promoter was obtained that also included three exons and two introns at the 5′ end of the *ABP1* gene. It was previously shown for the *AGAMOUS* and *GLABROUS1* genes and for the gibberellin biosynthetic gene *GA1* that transcription may be controlled by enhancers binding to intragenic sequences (Larkin and others 1993; Sieburth and Meyerowitz 1997; Chang and Sun 2002). The extended *ABP1* promoter conferred altered expression with respect to tissue specificity and developmental regulation compared to the 5′ upstream promoter region. The fact that the activities of the two promoter regions differed indicates that *cis* elements are present within the transcribed region downstream of the start codon. This possibility is supported by the finding that numerous transcription factor binding sites are predicted within this sequence. Such predictions are, however, preliminary and will have to be verified experimentally. Furthermore, it cannot be excluded that transcription factor binding sites that are not predicted exist downstream or upstream of the fragment analyzed.

The expanded *ABP1* promoter was active in young, growing leaves and was inactive in fully expanded rosette leaves except at the hydathodes, supporting a role for ABP1 in growth regulation. Leaf growth was shown to result from auxin-mediated cell division and cell expansion. In tobacco and *Arabidopsis*, ABP1 acts at low auxin concentrations to mediate expansion of leaf cells (Chen and others 2001b; Braun and others 2008). At high auxin levels ABP1 mediates cell division (Braun and others 2008). Expression of the antibody fragment scFv12, which binds and inactivates ABP1, caused cell cycle arrest in BY2 cells (David and others 2007). *ABP1:GUS* expression was further observed in etiolated and de-etiolated hypocotyls of *Arabidopsis* seedlings. Auxin is a regulator of hypocotyl growth along with gibberellin, ethylene, and brassinosteroids (Vandenbussche and others 2005). Analysis of the *tir1-1afb1-3afb2-3afb3-4* mutant revealed that hypocotyl elongation induced by indole-3-acetic acid was regulated independently of the TIR1/AFB family. It was proposed that growth may be regulated by ABP1 (Schenck and others 2010).

ABP1:GUS expression was high in the meristematic root tip and was observed in the root central cylinder, including the pericycle. Furthermore, developing lateral root primordia displayed *ABP1* promoter activity reminiscent of *CycA2;1* expression, which was observed in lateral root primordia within 8 h of auxin treatment. Similarly, *CycB1;1* expression was induced in this tissue following auxin-mediated cell cycle activation during lateral root initiation in *Arabidopsis* (Himanen and others 2002). The similar expression patterns of cyclin genes and *ABP1* support a function of ABP1 in meristematic root cells. In accord with a function in the regulation of cell division, ABP1 was shown to control entry into the cell cycle at the G1/S phase transition via the CYCLIN/RETINOBLASTOMA pathway (Tromas and others 2009). Inactivation of ABP1 alters the region of *PLETHORA* (PLT) expression. The transcription factor PLT contributes to root stem cell maintenance. Regulation by ABP1 may involve members of the Aux/IAA transcription factor family, which in turn may regulate PLT. Because Aux/IAA repressors are known targets of TIR1/AFB, it is conceivable that ABP1 and TIR1/AFB signaling pathways converge in the *Arabidopsis* root meristem to regulate expression of growth-associated genes.

DR5 activity is a marker for the ARF-AuxRE-dependent auxin response (Ulmasov and others 1997), which is driven mostly by auxin receptors of the TIR1/AFB family (Dharmasiri and others 2005; Kepinski and Leyser 2005; Tan and others 2007). Hence, DR5 activity might be considered an indicator of TIR1/AFB-mediated auxin activity even though *TIR1/AFB* expression has not yet been directly compared to DR5 activity. A comparison between DR5-driven expression and *ABP1* expression in leaves and roots revealed that the patterns were largely overlapping but not identical, indicating that ABP1 acts in part independent of ARF-AuxRE-mediated gene regulation and that ABP1 and TIR1/AFB receptors likely have at least some non-redundant functions.

The EGFP-ABP1 fusion protein was localized predominantly to the ER, in line with the presence of a KDEL motif at the C-terminus of ABP1. These results confirm localization to the ER in *Arabidopsis*, as reported previously for maize ABP1 (Ray 1977; Jones and Herman 1993; Diekmann and others 1995; Henderson and others 1997). It was proposed that a minor fraction of ABP1 escapes to the plasma membrane to act as an auxin receptor at the apoplastic side, possibly through binding to a GPI-anchored protein (Diekmann and others 1995; Henderson and others 1997; Shimomura 2006). A role for ABP1 in endocytosis of clathrin-coated vesicles was reported recently,

supporting activity of ABP1 at the plasma membrane (Robert and others 2010).

The predominant presence of ABP1 in the lumen of the ER still lacks a functional explanation. Is ER-localized perception of an auxin signal through ABP1 physiologically relevant? And if so for which cellular response? Given the recent discovery of PIN transporters in the ER membrane, the auxin concentration in the ER might be high enough for auxin to bind to ER-localized ABP1 in vivo (Mravec and others 2009). However, the pH described for the ER is neutral (Wilson and others 1993) and therefore more alkaline than the pH optimum determined for auxin binding to ABP1 (Tian and others 1995). ABP1 of *Zea mays* has a narrow pH optimum, with optimal binding at pH 5.5 and a K_D value of about 6×10^{-8} M for the synthetic auxin 1-naphthalene acetic acid (Löbler and Klämbt 1985). On the other hand, local pH values in the ER close to the Golgi were reported at around pH 6.5 (Kim and others 1998), which would favor auxin binding to ABP1. Finally, the question remains: Does auxin influence the subcellular distribution of ABP1 between the ER and the cell surface, perhaps controlled through ABP1 itself? The EGFP-ABP1 construct, with its flexible linker between EGFP and the ABP1 coding sequences described here, may produce functional ABP1 and may thus be a promising tool for addressing this point.

Acknowledgments Support of this work by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

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