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Altered Expression of Auxin-binding Protein 1 Affects Cell Expansion and Auxin Pool Size in Tobacco Cells

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Abstract

Auxin-binding protein 1 (ABP1) has an essential role in auxin-dependent cell expansion, but its mechanisms of action remain unknown. Our previous study showed that ABP1-mediated cell expansion is auxin concentration dependent. However, auxin distribution in plant tissue is heterogeneous, complicating the interpretation of ABP1 function. In this study, we used cells in culture that have altered expression of *ABP1* to address the mechanism of ABP1 action at the cellular level, because cells in culture have homogeneous cell types and could potentially circumvent the heterogeneous auxin-distributions inherent in plant tissues. We found that cells overexpressing ABP1 had altered sensitivity to auxin and were larger, with nuclei that have undergone endoreduplication, a finding consistent with other data that support an auxin extracellular receptor role for ABP1. These cells also had a higher free auxin pool size, which cannot be explained by altered auxin transport. In cells lacking detectable ABP1, a higher rate of auxin metabolism was observed. The results suggest that ABP1 has, beyond its proposed role as an auxin extracellular receptor, a role in mediating auxin availability.

Key words: ABP1; Auxin; Auxin conjugation; Cell elongation; Cell expansion

Introduction

Auxin regulates cell elongation, division, differentiation, and morphogenesis. Recently, transport inhibitor response 1 (TIR1), an F-box protein, has been proposed to be an intracellular auxin receptor (Dharmasiri and others 2005a, 2005b; Kepinski and Leyser 2005). This raises the long-standing question: what is the function of auxin-binding protein 1 (ABP1)? ABP1 is a proposed auxin receptor for some of the various auxin-mediated responses (Yamagami and others 2004). ABP1 purified from maize binds auxins in a saturable manner under defined conditions (Ray and others 1977; Lobler

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and Klambt 1985; Napier and others 1988; Jones and Venis 1989), and the binding profile for ABP1 correlates with the potency of auxin to elicit cell elongation (Ray and others 1977; Edgerton and others 1994; Rescher and others 1996), although this correlation is not without exception.

Substantial evidence from electrophysiological, biochemical, cytological, and molecular genetic studies all point to a role for ABP1 in mediating auxin-induced expansion. Auxin causes a rapid hyperpolarization of the plasma membrane of tobacco protoplasts as a result of changes in proton and potassium flux (Thiel and others 1993; Blatt and Thiel 1994). Antibodies to ABP1 alter the hyperpolarization response (Barbier-Brygoo and others 1989, 1991; Ruck and others 1993; Thiel and others 1993; LeBlanc and others 1999) and protoplast swelling (Steffens and others 2001; Yamagami and others 2004) in a manner consistent with a role for an extracellular receptor. Expression of maize ABP1 in tobacco leaves also alters potassium flux, and ABP1 abundance in guard cells correlates with auxin sensitivity as determined by auxin-induced K^+ flux (Bauly and others 2000). Ectopic, inducible expression of *Arabidopsis ABP1* in tobacco leaves confers inducible, auxin-dependent cell expansion in cells normally lacking auxin responsiveness, suggesting that auxin and ABP1 are growth limiting (Jones and others 1998; Chen and others 2001a). The change in auxin responsiveness at different positions in the lamina as leaves mature strictly correlates with the corresponding change in ABP1 abundance (Chen and others 2001a). Loss-of-function *abp1* mutants are unable to develop beyond an auxin-mediated step during embryogenesis (Chen and others 2001b). *Arabidopsis abp1* null mutant embryos arrest at a time during embryogenesis when cell expansion normally drives the transition from globular to heart-stage. Antisense suppression of ABP1 in BY-2 cells blocks auxin-induced cell elongation but has little or no direct effect on auxin-induced cell division (Chen and others 2001a, 2001b).

ABP1 is found predominantly in the lumen of the endoplasmic reticulum, but can also be detected throughout the endomembrane system (Jones and Herman 1993; Henderson and others 1997) and at the plasma membrane/cell wall interface (Deikman and others 1995). The unique localization of ABP1 does not yield any clues concerning its mode of action. Despite the preponderance of evidence consistent with ABP1's proposed receptor function, the role of ABP1 in cell expansion and the mechanisms by which its activity is mediated remain unknown. Here, to explore the role of ABP1 in cell expansion and to study how ABP1 might function, we utilize tobacco cells that overexpress or underexpress *ABP1* (Bauly and others 2000; Chen and others 2001a, 2001b). We provide evidence that in addition to its proposed role as an auxin extracelluar receptor, ABP1 may have a role in mediating auxin availability.

Materials and Methods

Tobacco Callus Culture

Leaf pieces of tobacco (*Nicotiana tabacum* cv Samsun) were previously used to generate transgenic lines constitutively overexpressing wild-type maize ABP1, mutated maize ABP1 (C-terminal ER-retention motif of maize ABP1 were mutated from KDEL to KEQL), or the empty expression vector (Bauly and others 2000). To obtain cells in culture, tobacco calli were generated from leaf strips of these transgenic lines. Calli were maintained on MS medium (pH 5.7) containing 3% sucrose and 0.6% phytoagar, supplied with 1 mg/l 1-naphthaleneacetic acid (NAA) (5.37 μM) and 0.5 mg/l 6-benzylaminopurine (6-BA) (2.22 μM) under continuous light at 23°C. Because no dramatic morphological difference was found between KDEL and KEQL plants, and because the KEQL mutation had no significant effect on ABP1 subcellular localization (Bauly and others 2000), we treated these two lines as similar with regard to ABP1 expression and localization. ABP1-expressing lines were denoted as MS1 and MS2, whereas the empty vector control line was denoted BC.

Cell Growth Measurement and Auxin Sensitivity Assay

Calli were subcultured every 30 days. Pieces of callus (~ 0.1 g) were taken from the top of 30-day-old calli and moved to fresh maintenance medium. The fresh weights of BC, MS1, and MS2 calli were measured 4, 7, 10, 14, 17, 21, 24, 28, and 31 days after subculture (three replicates). In the auxin sensitivity assay, calli were cultured in the maintenance media with a constant 6-BA concentration (2.22 μM) but with varying concentrations of NAA, differing by twofold in concentration in a series around the standard maintenance medium (set as 1×). Three replicates were done for each experiment. Experiments were repeated twice. Fresh weights of BC, MS1, and MS2 calli were measured 17 days after subculture, and the calli were photographed.

Reverse Transcription-Polymerase Chain Reaction

Total RNAs were isolated from BC, MS1, and MS2 calli 17 days after subculture using the TRIzol reagent (GIBCO BRL). cDNA was synthesized using 5 μg of total RNA by Oligo$(dT)_{20}$-primed reverse transcription, using THERMOSCRIPT RT (GIBCO BRL). The first strand of cDNA was used as a template for polymerase chain reaction (PCR) using Takara ExTaq polymerase (PANVERA, Madison, WI). Maize *ABP1* primers (5′-AGCCCGTGGCGCCTACCTC-3′ and 5′-TGCTGCTTCGAAGCAGTCCTC-3′, corresponding product size 553 bp) and tobacco *ACTIN* primers (5′-CCTCTTAACCCGAAGGCTAA-3′ and 5′-GAAGGTT GGAAAAGGACTTC-3′, corresponding product size 469 bp; accession number X63603) were added together in each PCR reaction.

Isolation of Nuclei and Flow Cytometric Analysis

Nuclei were extracted from tobacco calli using the procedures developed for tobacco leaves (Galbraith and others 1983; Chen and others 2001a). Calli from BC, MS1, and MS2 were sampled 17 days after subculture, excised, immediately chilled on ice, and chopped with a single-edged razor blade in a glass Petri dish containing a chopping buffer (pH 7.0, 4°C) of the following composition: 45 mM $MgCl_2$, 30 mM sodium citrate, 20 mM 3-(N-morpholino) propane sulfonate (MOPS), 0.1% Triton X-100 (Galbraith and others 1983; Chen and others 2001a). The nuclear suspensions were passed through 60-μm nylon mesh. Propidium iodide and RNAase were added to a final concentration of 100 μg/ml and 10 μg/ml, respectively. The samples were kept on ice prior to flow cytometric analysis. About 5000 stained nuclei for each sample were analyzed by flow cytometry (FACScan, manufactured by Becton-Dickinson Immunocytometry Systems) with a 488 nm light source from a 15 mW argon ion laser. The flow rate was adjusted to approximately 50 nuclei per second. Chicken red blood cells were used as an internal standard and for instrument alignment. The FACScan is interfaced to a Cicero data acquisition system (Cytomation, Inc.). Intact cells from BC, MS1, and MS2 calli were also stained with DNA dye 4′,6-diamidino-2-phenylindole (DAPI) to visualize nuclei using fluorescence microscopy.

Thymidine Incorporation

Calli from BC, MS1, and MS2 17 days after subculture were moved to maintenance medium without phytoagar, but with 1 μCi of ^{3}H-thymidine (three replicates). The calli were continuously cultured with shaking for another 12 h, washed 5 times with dH_2O, homogenized, centrifuged 1000 × g for 10 min, resuspended in 1 ml of 10% ice-cold trichloroacetic acid (TCA), and left on ice for 30 min. The pellets were washed twice with 5% ice-cold TCA, once with 100% ethanol, and solubilized in 0.1 M NaOH and 0.2% sodium dodecyl sulfate (SDS) at 37°C for 30 min. Radioactivity was measured by liquid scintillation, and ^{3}H-thymidine incorporation was expressed as DPM per 50 mg fresh weight of callus.

Auxin Analysis

We used the 3-indoleacetic acid (IAA) ELISA kit developed at the Nanjing Agricultural University. This ELISA kit has been commercially available for more than a decade. A similar kit is available from Sigma Chemicals (St. Louis, MO; product number PGR-3). The validation of such an immunoassay had been performed by both high performance liquid chromatography (HPLC) and gas chromatography-mass spectroscopy (GC-MS) analyses when the kit was originally established. In a standard procedure, both a recovery assay (using an internal control) and a dilution assay (making a series of sample extract dilutions) were performed, the first to ensure good sample recovery and the second to guarantee the absence of nonspecific inhibitors in the extracts. This IAA immunoassay has been used to measure IAA contents in various tissues (Chen and others 1996, 1997a, 1997b, 1998).

In this study, calli from BC, MS1, and MS2 were sampled 17 days after subculture, and lyophilized. Samples were powdered under liquid nitrogen and extracted in 2 ml of cold 80% (v/v) aqueous methanol overnight at 4°C with butylated hydroxytoluene (10 mg/l). The supernatant was collected after centrifugation at 10,000 × g (4°C) for 15 min, and passed through a C18 Sep-Pak cartridge (Waters Corporation, Milford, MA). The efflux was collected, and 300 μl of it was removed and dried in N_2 gas. The residue was dissolved in 200 μl of 100% methanol for methylation with freshly synthesized ethereal diazomethane. The solution was then dried under N_2 gas and redissolved in 300 μl of phosphate buffered saline (PBS) for IAA ELISA using a monoclonal antibody of high specificity for IAA methyl ester. The main steps are as follows: microtitration plates (Nunc, Denmark) were precoated overnight at 4°C with rabbit anti-mouse immunoglobulin. After blocking

with 0.2% BSA, the wells were coated with anti-IAA methyl ester monoclonal antibody in PBS (0.01 M, pH 7.4) at 37°C for 70 min. Authentic IAA methyl ester or sample was added 30 min before the addition of horseradish peroxidase–labeled IAA. After incubation at 37°C for 1 h, the wells were washed with PBS containing 0.05% Tween-20. Buffered enzyme substrate (H_2O_2 and orthophenylenediamine) was added, and the enzyme reaction was carried out in darkness at 37°C for 15 min. The reaction was terminated with 3 M H_2SO_4 and the absorbance was recorded at 490 nm. IAA was determined three times on the same extract, and samples were assayed in triplicate.

Auxin Uptake

Tobacco BY2 cells lacking detectable ABP1 (designated NAS1) and BY2 cells transformed with the corresponding empty vector have been described previously (Chen and others 2001a, 2001b). These cell lines as well as the wild-type, untransformed, BY2 cells were used for auxin uptake assay precisely as described by Delbarre and others (1996). Tobacco cells (~0.1 g) taken 4 days after transfer to fresh culture medium were washed as described by Delbarre and others (1996). Aliquots of approximately 50 mg of cells in 5 ml of uptake buffer (Delbarre and others 1996) were incubated with [^{3}H]-NAA or [^{14}C]-2,4-dichlorophenoxyacetic acid (2,4-D) (~500,000 DPM) for the indicated times and sampled by filtration and rapidly washed. Fresh weight of cells on filters were determined and used to normalize each sample. Radioactivity in cells was extracted in ethanol and measured directly by liquid scintillation fluid (toluene). Typically, 5% of the total DPM added was incorporated into cells. In parallel samples, 10 μM naphthylphthalamic acid (NPA) was included to block NAA efflux. Cells were extracted in 95% ethanol, and cell debris was removed by centrifugation. The supernatant (10 μl) was analyzed by TLC using silica plates and benzene:acetic acid: anhydrous methanol 90:8:16 as a solvent system.

Results

Tobacco Callus Cells Constitutively Expressing Maize ABP1 Are Larger

We previously showed that cells lacking detectable ABP1 (designated NAS1) have smaller cell volumes and have lost auxin-inducible growth promotion (Chen and others 2001a, 2001b). These antisense data have been extended by generating two independent tobacco cell lines from leaf strips of tobacco plants constitutively overexpressing maize *ABP1* (designated MS1 and MS2). A control line (designated BC) from plants transformed with the corresponding empty expression vector was also produced. MS1 and MS2 calli appear to be greener than BC callus, and they may contain more chlorophyll. *ABP1* transcript levels in these cell lines were checked by reverse transcription-polymerase chain reaction (RT-PCR) (Figure 1). We chose to use fresh weight (FW) as our measurement of callus growth. The callus was maintained in a medium containing both auxin and cytokinin. Under this condition, the callus grows to a mass of cells or clusters of cells, in which individual cells are very difficult to separate. Technically, it is not possible to count the cell number in a given callus. The generation of suspension cells from BC, MS1, and MS2 calli was not successful. Therefore, we chose to use fresh weight to monitor the callus growth. When cultured in MS medium supplied with 1 mg/l NAA (5.37 μM) and 0.5 mg/l 6-BA (2.22 μM), each line produced calli of a similar size growing at similar rates (Figure 2a–d). However, the cell surfaces of MS1 and MS2 cells were significantly larger than BC control cells (Figure 2e), consistent with the earlier finding that overexpression of *Arabidopsis ABP1* in tobacco plants caused increased cell size (Jones and others 1998).

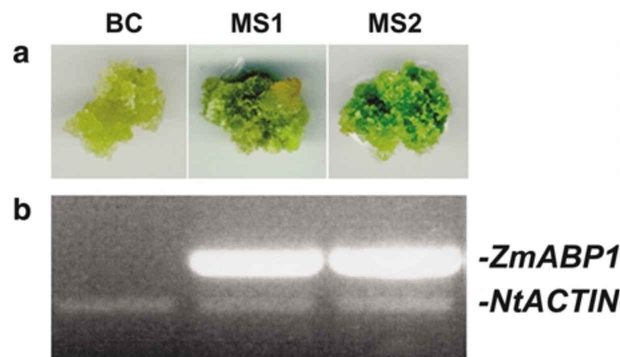
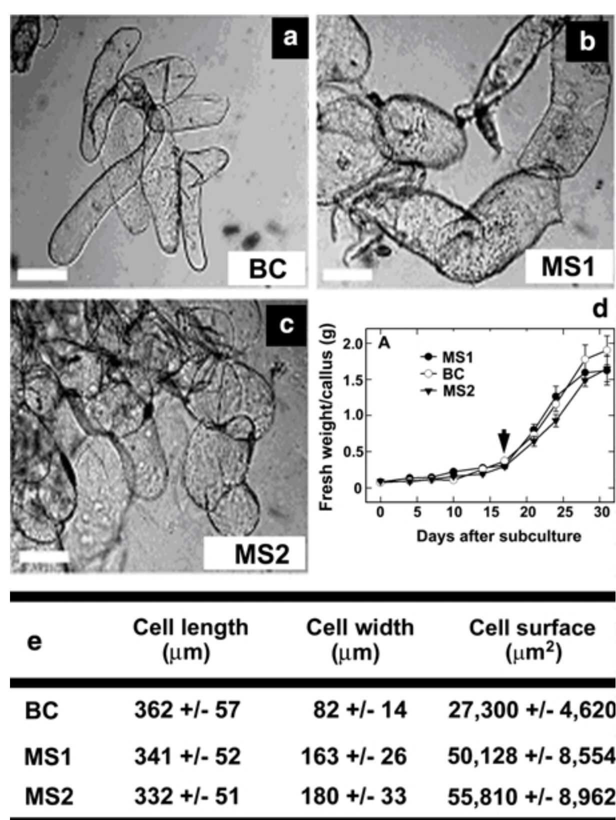


Figure 1. Overexpression of maize *ABP1* in tobacco callus cells. (**a**) The morphology of tobacco callus cells overexpressing maize *ABP1*. BC, callus cells transformed with the empty expression vector. MS1, and MS2 are callus cells transformed with the maize *ABP1* as described in *Material and Methods*. (**b**) Reverse transcription-polymerase chain reaction (RT-PCR) analysis of maize *ABP1* transcript in tobacco callus cells. As a control, actin primers that amplify a 469-bp product were added together with maize *ABP1* primers in each PCR reaction.



e	Cell length (μm)	Cell width (μm)	Cell surface (μm²)
BC	362 +/- 57	82 +/- 14	27,300 +/- 4,620
MS1	341 +/- 52	163 +/- 26	50,128 +/- 8,554
MS2	332 +/- 51	180 +/- 33	55,810 +/- 8,962

Figure 2. Cell size and growth curve of tobacco cells overexpressing maize *ABP1*. Shown here are callus cells from BC (**a**), MS1 (**b**), and MS2 (**c**) 17 days after subculture. Scale bars = 100 μm. (**d**) The growth curve of tobacco callus cells overexpressing maize *ABP1*, measured as fresh weight increase over time. Data shown are the means ± SEM of three replicates. Seventeen days after subculture (arrows) is the time point that was used for subsequent sampling. (**e**) Cell length, width, and surface area of tobacco callus cells lines. Data shown are mean ± SEM of at least 50 cells.

Tobacco Cells Constitutively Expressing Maize ABP1 Undergo Endoreduplication

Large cell sizes have often been correlated with endoreduplication (Lur and Setter 1993; Valente and others 1998). Consequently, chromosomal replication was monitored in the nuclei of MS cell lines by flow cytometry and found to be dramatically advanced over BC control cells. Approximately 90% of cells overexpressing *ABP1* were at the 8C nuclear stage, indicating that these cells were undergoing endoreduplication (Figure 3). In control cells at this developmental stage about 47% of nuclei were at the G2 nuclear stage (4C), and only around 6% of nuclei were at the 8C nuclear stage.

Confirmation of endoreduplication in MS cells was made by DAPI staining. MS1 and MS2 cells contained larger nuclei with stronger fluorescence than nuclei in BC cells (Figure 3), and only occasionally were binucleate cells observed. The larger nuclei of MS1 and MS2 cells is consistent with a two- to threefold increase in DNA synthesis as shown by ^{3}H-thymidine incorporation (Figure 4).

Because callus cells of MS1 and MS2 are significantly larger, our data are consistent with overexpression of ABP1 promoting cell expansion (Figure 2) over cell division. DNA synthesis in cells overexpressing ABP1 increased by endoreduplication (see Figure 3), consistent with our previous finding that cell size correlates with nuclei phases (Chen and others 2001a).

Auxin Sensitivity Is Altered in ABP1 Overexpressing Cells

No significant difference in fresh weight accumulation was found between MS and control cells measured 30 days after subculture onto 5.37 μM NAA (Figure 2d). Because tobacco cells require auxin for proliferation in culture (Murashige and Skoog 1962), we tested whether auxin sensitivity was altered for proliferation in cells overexpressing *ABP1*.

Cells were cultured in media with a range of auxin concentrations (0–85.92 μM NAA), but with a fixed cytokinin concentration (2.22 μM 6-BA). Both *ABP1* overexpressing callus lines and the control callus line displayed the same maximum growth at an NAA concentration around 5.37 μM, but MS1 and MS2 lines displayed dose responsiveness different from control cells below this optimum concentration (Figures 5, 6). Below the optimum concentration of NAA for growth, MS cells proliferate more than control cells. To avoid misinterpretation, we measured the percentage of dry mass of the total fresh weight of calli. The average percentage of dry mass of the total fresh weight for MS1, MS2, and BC cells were 4.72 ± 0.7%, 4.83 ± 0.8%, and 3.05 ± 0.5%, respectively, indicating that the increase in biomass of MS1 and MS2 cells was not due to a substantially higher retention of water. These results demonstrate that MS1 and MS2 cell proliferation is more sensitive to low concentrations of exogenous NAA.

In the absence of exogenous auxin, control BC cells turned brown and were no longer viable by day 17, whereas MS1 and MS2 cells survived and proliferated beyond approximately 42 days (data not shown).

The increase in fresh weight could be due to increased cell expansion, increased cell division, or

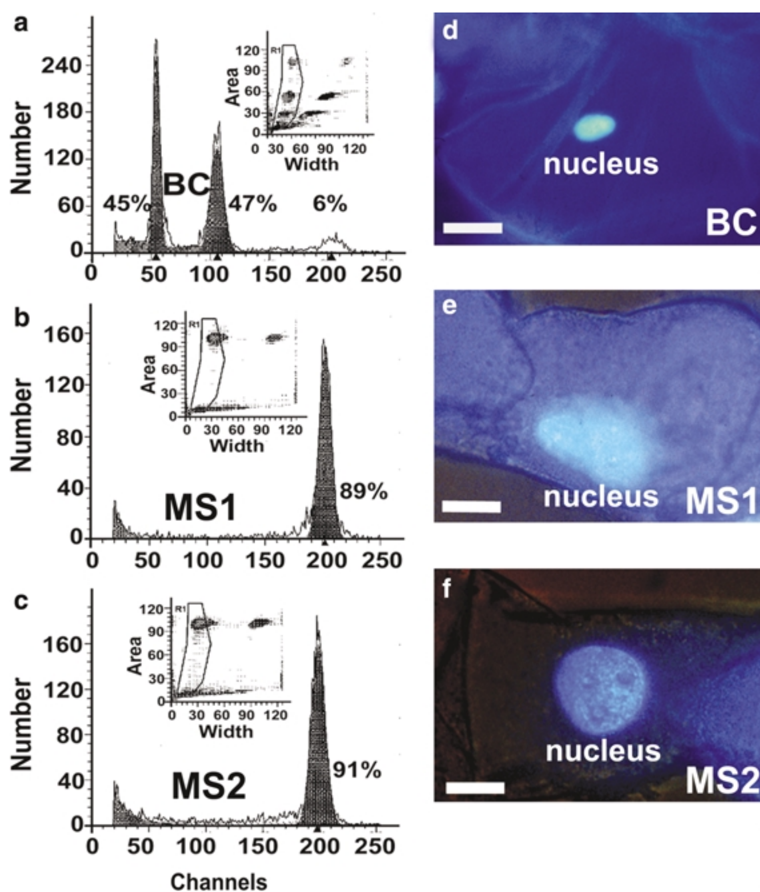


Figure 3. Flow cytometric analysis of tobacco callus cells overexpressing maize *ABP1* transgene. Nuclei were isolated from BC (**a**), MS1 (**b**), and MS2 (**c**) callus cells 17 days after subculture. Nucleus stages were measured by FACScan as described in *Material and Methods*. Shown are representative results from one of three independent experiments. The nuclei from BC (**d**), MS1 (**e**), and MS2 (**f**) callus cells were stained with DAPI and visualized using fluorescence microscopy. Scale bars = 25 μm.

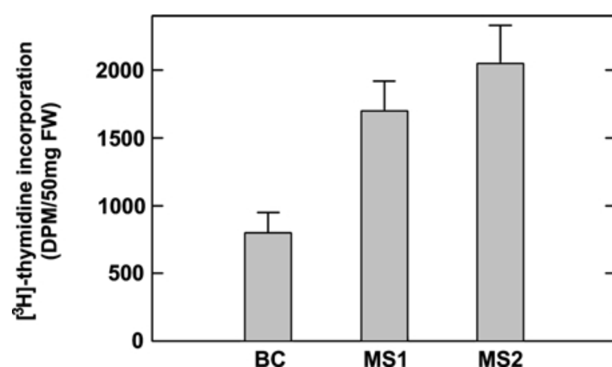


Figure 4. DNA synthesis in tobacco callus cells overexpressing maize *ABP1*. DNA synthesis was measured by ^{3}H-thymidine incorporation, and expressed as DPM per 50 mg fresh weight of callus as described in *Material and Methods*. Data shown are mean ± SEM of three replicates.

both. Because we were unable to directly count the cell number in these calli, it is not yet known if overexpression of ABP1 also promotes cell division in MS1 and MS2 callus cells.

A direct measurement of endogenous free auxin revealed that MS1, MS2, and BC cells contained 4.3 ± 0.41, 4.7 ± 0.39, and 2.1 ± 0.22 nmol/g FW, respectively, indicating that ABP1-overexpressing cells contain twice as much free auxin as control cells. One interpretation of the altered NAA responsiveness of ABP1-overexpressing cells is that their endogenous auxin pools are elevated. However, this conclusion is not consistent with the dose-responsiveness data. A larger endogenous auxin pool would be expected to shift the dose–response curve to the left. Although the dose–response shows an approximate twofold shift in MS1 and MS2 cells below the optimum for control cells, above this optimal concentration, the auxin responsiveness of MS cells was statistically unchanged compared to the control.

ABP1 Is Not Required for Auxin Transport

One mechanism by which the auxin content of cells might be increased is by altered auxin transport activities, particularly by reduction of IAA efflux. Because of the growth nature of callus cells (a mass of cells or clusters of cells), it is not technically possible to carry out auxin uptake or metabolism studies in these callus cells. Because of the advantage of the suspension cell system (Delbarre and

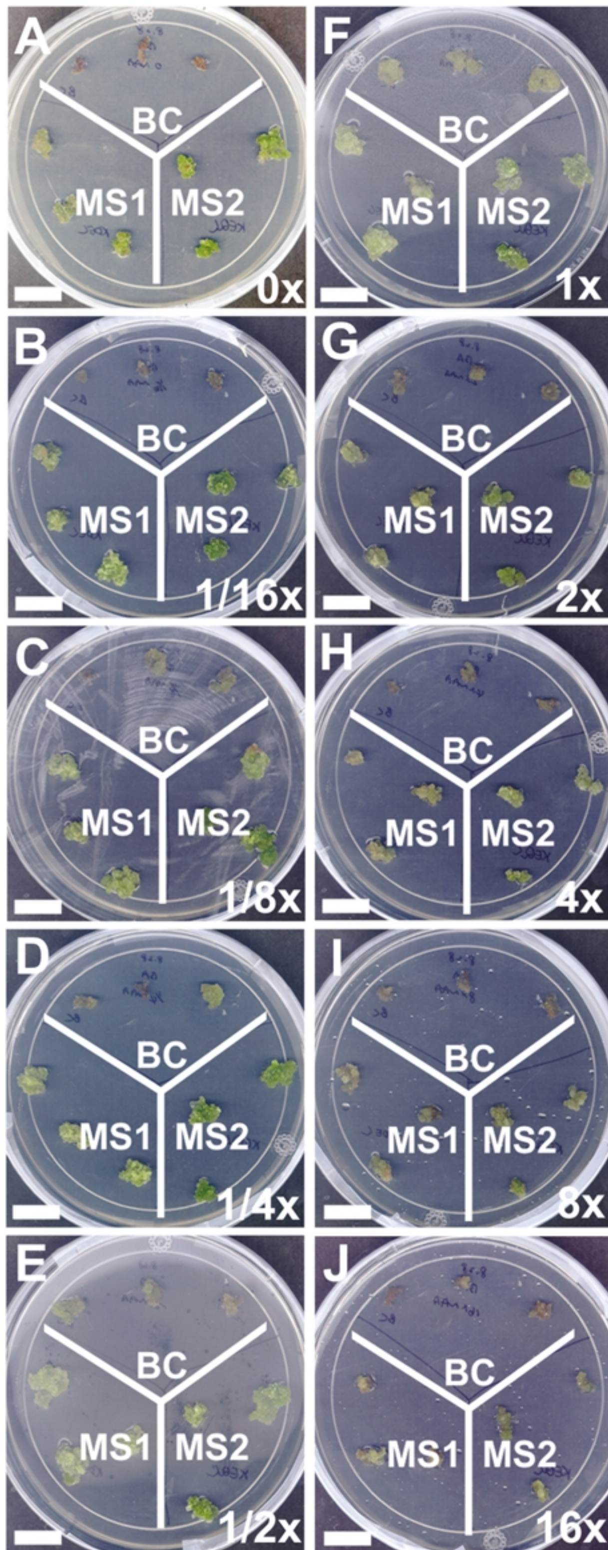


Figure 5. Morphology of tobacco callus cells overexpressing maize *ABP1* in response to different concentrations of auxin. Callus cells of BC, MS1, and MS2 were cultured in the maintenance media with 2.22 μM 6-BA but with varying concentrations of NAA, in a twofold concentration series around the NAA concentration (5.37 μM) of the standard maintenance medium (set as 1×). The final NAA concentrations in the media were 0 (**A**), 0.34 (**B**), 0.67 (**C**), 1.34 (**D**), 2.69 (**E**), 5.37 (**F**), 10.74 (**G**), 21.48 (**H**), 42.96 (**I**), and 85.92 μM (**J**), respectively.

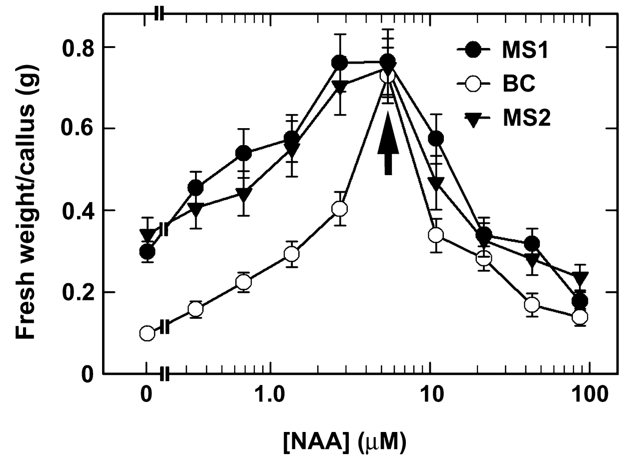


Figure 6. Auxin sensitivity of tobacco callus cells overexpressing maize *ABP1*. Callus cells of BC, MS1, and MS2 were culture in the maintenance media with 2.22 μM 6-BA, but with varying concentrations of NAA. Seventeen days after subculture, the fresh weight of each callus was measured and plotted over NAA concentration. The NAA concentrations were set in a logarithmic scale in the figure. The arrow indicates the concentration of NAA in maintenance medium. Data shown are mean ± SEM of three replicates.

others 1996), we used the ABP1 antisense suspension cell line NAS1 (Chen and others 2001a, 2001b) for these experiments. Net [^{3}H]-NAA or [^{14}C]-2,4-D accumulation was measured in NAS1 and compared with that in control cells (Figure 7a). Rapid auxin uptake and NPA-sensitive efflux activity were measured in all cell lines, but no differences were found between antisense and control cells (Figure 7a). In addition, there was no significant difference in the rates of [^{14}C]-2,4-D uptake between NAS1 and control cells (data not shown). The data together suggest that ABP1 is not essential for auxin uptake or efflux.

Cells with Reduced ABP1 Levels Have an Increase in Auxin Metabolism

Another possibility for the increased free IAA content in the MS cells might be altered auxin metab-

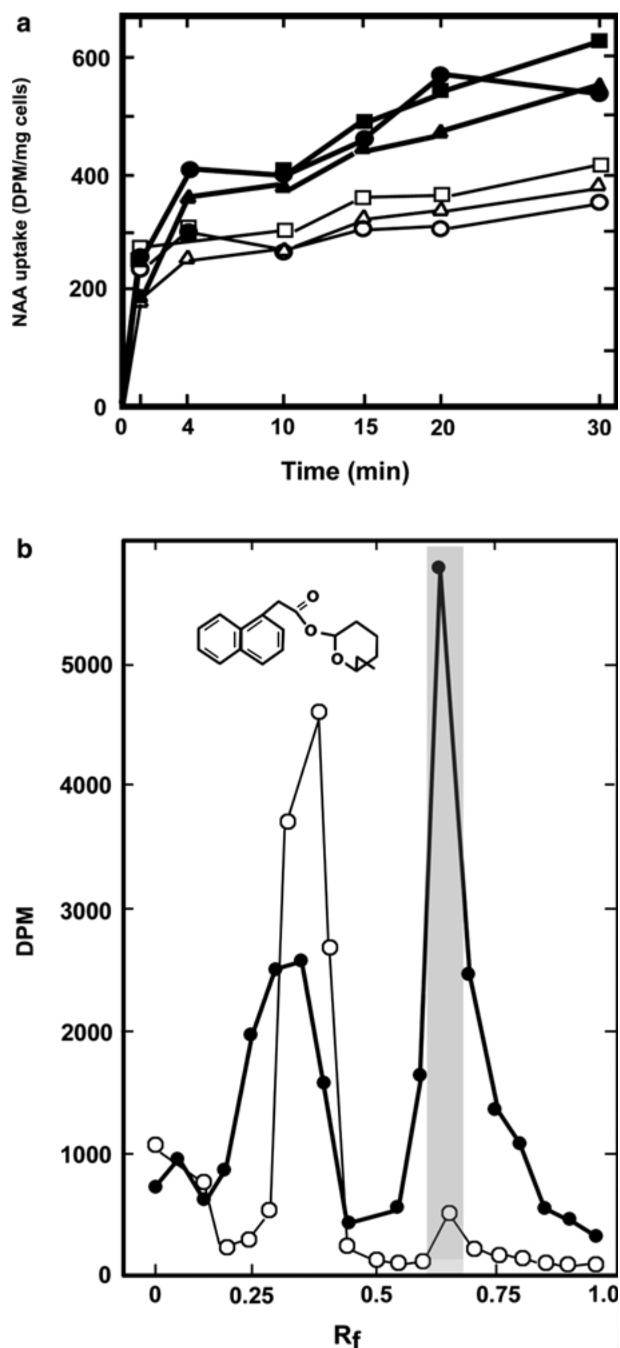


Figure 7. Net auxin uptake and auxin metabolism analyses in cells lacking detectable *ABP1*. (**a**) Net auxin uptake analysis. NAS1 (squares), wild-type, untransformed cells (circles), and cells transformed with empty expression vector (triangles) were incubated in the presence of [^{3}H]-NAA, with (open symbols) or without (closed symbols) the auxin transport inhibitor NPA (10 μM), and harvested at the times indicated. Radioactivity in the cells was measured by liquid scintillation counting. Auxin uptake is expressed as DPM per mg fresh weight of cells. (**b**) NAA catabolism/modification analysis. NAS1 (open circle) and cells transformed with empty expression vector (closed circle) were incubated in the presence of [^{3}H]-NAA, and harvested after 15 min. The radioactivity was extracted, and subjected to thin layer chromatography (TLC) as described in *Materials and Methods*. Both cell lines contained a radioactive peak corresponding to the position of authentic [^{3}H]-NAA (gray column), plus an additional peak corresponding to the 1-naphthyl-1-acetyl)-b-D-glucopyranose NAA conjugate (NAGP, Delbarre and others 1994) shown in the inset.

olism. To address this directly, the fate of exogenously added auxin in cells with ABP1 and cells lacking detectable ABP1 (NAS1) was determined. For this experiment, we used NAA. There were three reasons that we used synthetic auxin NAA instead of native auxin IAA for our metabolism experiment. First, it was critical that we build on published work. Delbarre and others (1994, 1996), followed the metabolism of NAA, not IAA in BY2 cells, and we have followed up on their finding. They identified the main product and characterized its behavior in thin layer chromatography (TLC), thus enabling us to extend their observation by determining the role of ABP1 in auxin metabolism. Second, radioactive IAA is not stable. All commercial sources to date are not radiopure despite their specifications. Finally, it has been reported that IAA and NAA are identical in their mode of action with regard to gene expression (Pufky and others 2003).

Consequently, NAA, not IAA, has become the standard auxin for cultured cells. Cells were incubated in the presence of [^{3}H]-NAA, harvested at several time points, and the radioactivity extracted. Samples were subjected to TLC (Figure 7b). After 15 min, both cell lines contained a radioactive peak that corresponded to the position of authentic [^{3}H]-NAA, plus an additional peak corresponding to the NAA conjugate, 1-naphthyl-1-acetyl-b-D-glucopyranose (NAGP, Delbarre and others 1994), indicating that cells lacking detectable ABP1 have accelerated NAA metabolism.

Discussion

This report extends published data describing the effects of altered *ABP1* expression and suggests that one of the effects of ABP1 action is exerted on the available pool size of free IAA in tobacco cells. It has been shown previously that higher ABP1 levels cause auxin-dependent increases in cell size and auxin-regulated K^+ flux and that reduction in ABP1 reduces

auxin-dependent cell expansion (Jones and others 1998; Bauly and others 2000; Chen and others 2001a, 2001b). We have shown that increased levels of ABP1 directly or indirectly cause a higher free auxin pool size and that this observation is consistent with auxin-related phenotypes described here and elsewhere. For example, it is known that elevated auxin induces endoreduplication (Lur and Setter 1993; Valente and others 1998; Mishiba and others 2001; Quelo and others 2002), a phenotype now also shown to be present in *ABP1*-overexpressing MS cells (Figure 3).

We have also shown that reduced levels of ABP1 in cells were associated with more rapid conjugation of applied NAA, suggesting that ABP1 has a role in mediating auxin availability. Although the mechanism is unknown, our data do not support a role of ABP1 in mediating auxin uptake. ABP1 might act to sequester or compartmentalize free auxins from modifying enzymes; however, it is unclear into which compartment auxin could be sequestered or compartmentalized. It is known that ABP1 does not bind auxin in the endoplasmic reticulum (ER), because the ER lumen has an estimated pH value of 7.0, whereas the ABP1-auxin has a sharp binding optimum of pH 5.5 (Tian and others 1995).

Therefore, it appears unlikely that the auxin sequestration compartment can be the ER. Alternatively, ABP1 could alter the free auxin pool size by regulation of enzyme activities rather than direct sequestration. It is also possible that ABP1 could bind auxin conjugates and the conjugates could then be cleaved by hydrolases. One auxin hydrolase, IAR3 (Davies and others 1999), is ER localized. Consequently, the long-term responses to altered ABP1 levels (endoreduplication, cell culture accumulation, and so on) could be explained in terms of either altered free auxin pool sizes or altered receptor activity. However, more rapid responses such as membrane ion fluxes and protoplast swelling (see, for example, Ruck and others 1993; Yamagami and others 2004) are unlikely to be attributed to auxin sequestration alone, because, in these cases, interference with ABP1 activity removed auxin responsiveness rather than shifted the dose–response curves. Regardless of a direct or indirect effect, the data presented above introduce a novel and additional action mediated by ABP1 without contradicting the evidence supporting an extracellular receptor role for ABP1.

Our finding that overexpression of ABP1 promotes cell expansion in culture cells is consistent with that in intact plants (Jones and others 1998; Chen and others 2001a). By using the cells in culture, we were able to further measure the effect of ABP1 on auxin uptake and metabolism, and provide evidence that ABP1 may mediate auxin availability. Although we are cautious in our interpretations of the data, because of the use of a rather artificial system, our findings offer a clue to how ABP1 might function in an intact plant.

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