ABP1 is required for organized cell elongation and division in *Arabidopsis* embryogenesis

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To directly address the function of a putative auxin receptor designated ABP1, a reverse genetic approach was taken to identify and characterize *ABP1* mutant alleles in *Arabidopsis*. A homozygous null mutation in *ABP1* confers embryo lethality. Null mutant embryos develop normally until the early stages of the globular embryo but are unable to make the transition to a bilaterally symmetrical structure because cells fail to elongate. Cell division was also aberrant both in the suspensor and embryo proper. Antisense suppression of *ABP1* in tobacco cells causes slow proliferation and eliminates auxin-induced cell elongation and reduces cell division. The complete lack of auxin-inducible elongation in individual cells confirms the results observed in embryos, indicates a cell autonomous function, and, taken together with biochemical evidence that ABP1 binds auxins, suggests that ABP1 mediates auxin-induced cell elongation and, directly or indirectly, cell division.

[*Key Words*: Auxin; auxin-binding protein 1; embryogenesis; BY-2; cell elongation; cell division]

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The plant hormone class, auxins, regulates three essential cellular fates in morphogenesis, elongation, division, and differentiation. However, the receptor(s) mediating any auxin action is unclear. The best known candidate for an auxin receptor mediating auxin-regulated cell expansion is auxin-binding protein 1 (ABP1), discovered 25 years ago (Batt et al. 1976; Ray et al. 1977) and characterized extensively since then. ABP1 is a low abundance protein found predominantly within the endoplasmic reticulum only in cells of aerial tissues that have the capacity to expand by auxin. ABP1 has been shown to bind auxins at concentrations corresponding to physiological concentrations for their individual activities (Jones and Venis 1989). An informative array of compounds was used to propose a model of the auxin-binding site indicating the structural constraints expected for a receptor (Edgerton et al. 1994). Antibodies directed against ABP1 as well as ABP1 peptide mimetics modulate auxin-regulated plasma membrane hyperpolarization, the earliest event in auxin-induced cell wall loosening (Barbier-Brygoo et al. 1989, 1991; Ruck et al. 1993; Thiel et al. 1993; LeBlanc et al. 1999). Furthermore, ectopic and inducible expression of ABP1 confers auxin-dependent cell expansion in cells normally lacking auxin responsiveness (Jones et al. 1998). Whereas the biochemical and molecular genetic evidence for receptor function is compelling, direct genetic evidence of function has been lacking. In particular, the Abp$^-$ phenotype has been elusive.

The *Arabidopsis* genome contains only one *ABP1* gene (Palme et al. 1992). Recently, a method to screen for insertion mutants in *Arabidopsis* has been developed to isolate genetic knockouts (Krysan et al. 1996, 1999). This reverse genetic approach has been used in this study to examine the loss-of-function state for the *ABP1* gene. In addition, the observed phenotype in *Arabidopsis* prompted hypotheses on ABP1 function that were tested by use of a simpler single cell system.

Results

The abp1 *insertion allele is a null mutation and confers lethality*

By use of a PCR-based strategy, one mutant allele was identified and shown by direct sequencing to harbor T-DNA in the predicted first exon of the *ABP1* gene (Fig. 1A). The T-DNA insertion was at a site that was 51 bp 3′ to the start codon but before the cleavage site for the signal peptide.

No individual homozygous at the *ABP1* locus was found from a large screen of T2 (data not shown) and T3 plants (Fig. 1B,C), suggesting that this mutation in its homozygous state is lethal. Southern analysis at high

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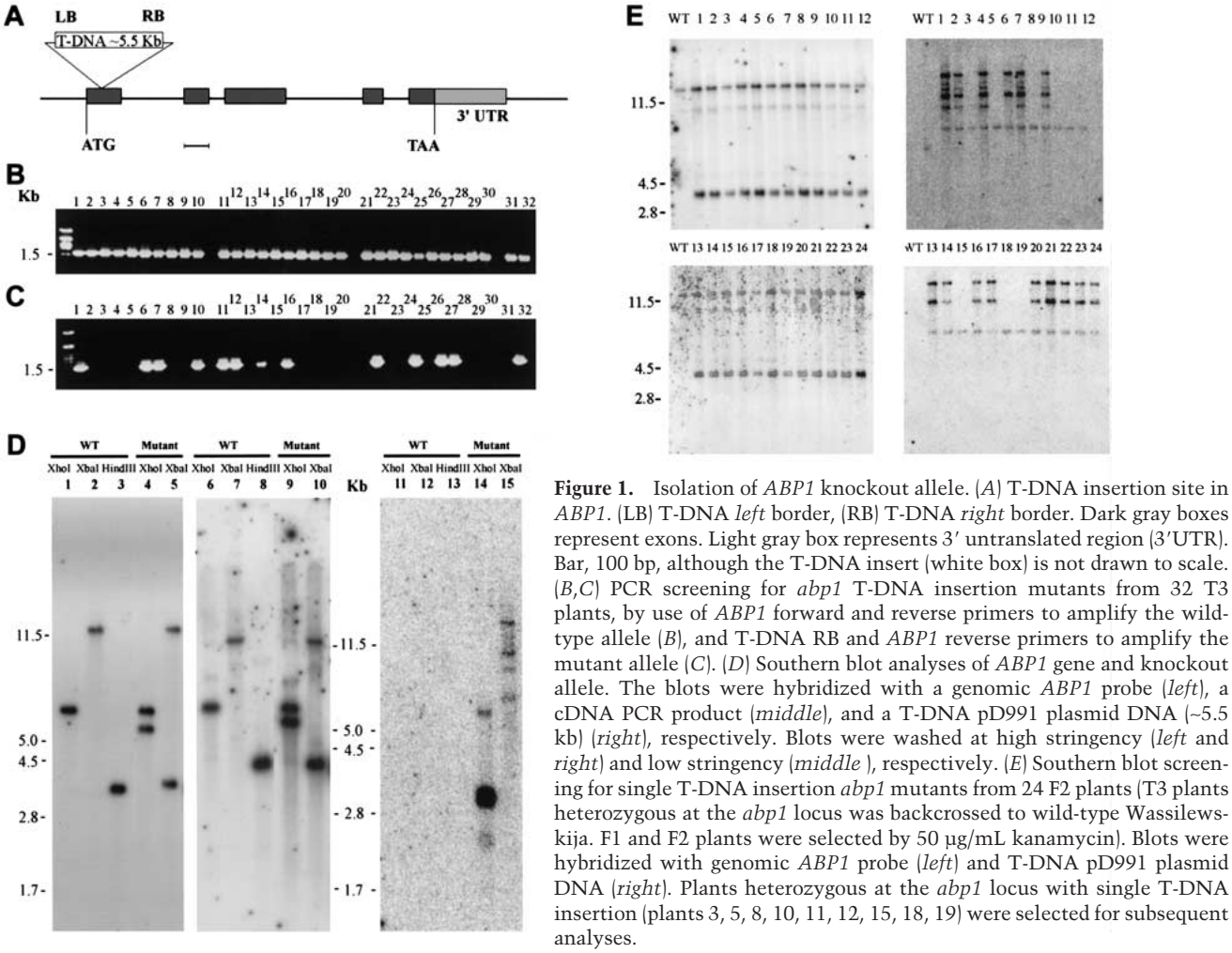

Figure 1. Isolation of *ABP1* knockout allele. (*A*) T-DNA insertion site in *ABP1*. (LB) T-DNA *left* border, (RB) T-DNA *right* border. Dark gray boxes represent exons. Light gray box represents 3′ untranslated region (3′UTR). Bar, 100 bp, although the T-DNA insert (white box) is not drawn to scale. (*B*,*C*) PCR screening for *abp1* T-DNA insertion mutants from 32 T3 plants, by use of *ABP1* forward and reverse primers to amplify the wild-type allele (*B*), and T-DNA RB and *ABP1* reverse primers to amplify the mutant allele (*C*). (*D*) Southern blot analyses of *ABP1* gene and knockout allele. The blots were hybridized with a genomic *ABP1* probe (*left*), a cDNA PCR product (*middle*), and a T-DNA pD991 plasmid DNA (~5.5 kb) (*right*), respectively. Blots were washed at high stringency (*left* and *right*) and low stringency (*middle*), respectively. (*E*) Southern blot screening for single T-DNA insertion *abp1* mutants from 24 F2 plants (T3 plants heterozygous at the *abp1* locus was backcrossed to wild-type Wassilewskija. F1 and F2 plants were selected by 50 µg/mL kanamycin). Blots were hybridized with genomic *ABP1* probe (*left*) and T-DNA pD991 plasmid DNA (*right*). Plants heterozygous at the *abp1* locus with single T-DNA insertion (plants 3, 5, 8, 10, 11, 12, 15, 18, 19) were selected for subsequent analyses.

and low stringency by use of genomic *ABP1* and its cDNA as probes respectively confirmed that the WS ecotype harbored a single *ABP1* gene and the insertion segregated with this gene in the mutant (Fig. 1D). Backcrossing to wild-type Wassilewskija enabled isolation of plants with single T-DNA insertions (Fig. 1E, plants 3, 5, 8, 10, 11, 12, 15, 18, and 19) linked with the kanamycin marker (kan^R:kan^S = 2:1) and tagged to the *ABP1* gene (Fig. 1E), and these were used for further characterization.

The absence of homozygous null alleles in the screen and the observed kan^R segregation ratio suggested that lethality was embryonic, therefore, immature seeds were examined within each silique. Because *Arabidopsis* seeds mature synchronously within each silique, it is possible to score segregating individuals having aberrant development (Errampalli et al. 1991). The siliques from wild-type and mutant plants were normal (Fig. 2A). However, 8–12 d after flower opening, ~25% of immature seeds from mutant plants heterozygous at the *abp1* locus were distinguishable by color (Fig. 2B,C). The embryos of these abnormally white, immature seeds were arrested at the globular stage (Fig. 2D), whereas those of the green immature seeds had already reached the mature cotyledon stage (Fig. 2, cf. D with E). At a point when wild-type seeds were fully mature, the segregating white seeds turned brown and lost germination capacity as described for tagged, embryonic-lethal mutants (Errampalli et al. 1991). This shows that the mutation linked to kan^R confers embryo lethality.

To determine whether the embryo defect was exclusively caused by a single T-DNA insertion in the *ABP1* gene, genetic complementation was carried out by cotransforming mutants, heterozygous at the *ABP1* locus, with CaMV *35S*::*ABP1* and *35S*::*Bar*. Basta-resistant plants were selfed and the seed lethality scored. Within siliques of T2 plants, white seeds segregated from green seeds at the expected segregation ratio (1:15) for rescue by the transgene (Table 1). Rescued plants homozygous at the *abp1* locus were analyzed further by use of PCR to genotype and ascertain the presence of the transgene. Those segregating 1:15 white to green seed were shown to be homozygous at the *abp1* locus and hemizygous at the *35S*::*ABP1* transgene locus. BASTA resistance seg-

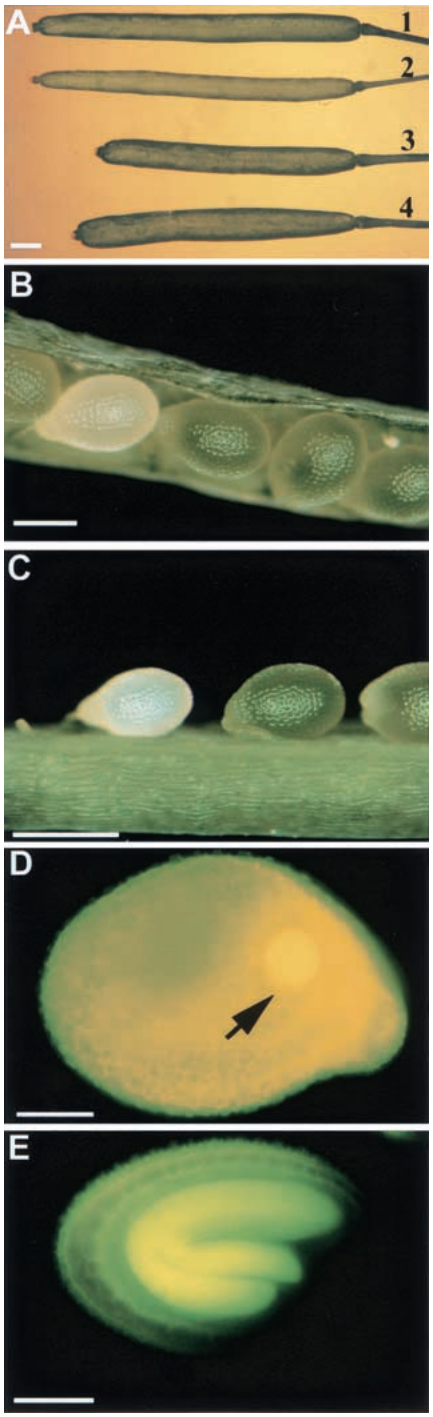

Figure 2. Immature seed segregation in plants heterozygous at the *abp1* locus. (*A*) Siliques of wild-type Wassilewskija and Landsberg *erecta* plants (1 and 3) and mutant plant heterozygous at the *abp1* locus in the corresponding ecotypes (2 and 4). (*B*,*C*) Immature seeds segregate within single silique of plants heterozygous at the *abp1* locus. (*D*,*E*) Microscopy image of white (*D*) and green (*E*) immature seeds from single silique of mutant plant heterozygous at the *abp1* locus in *B* and *C*. Images were obtained by use of a 490-nm excitation filter. Arrow in *D* indicates a globular *abp1* embryo of the same developmental age as the wild-type embryo shown in *E*. Scale bars, 500 μm in *A–C*, and 100 μm in *D* and *E*.

Table 1. *abp1 mutant rescued by transformation with CaMV 35S::ABP1*

	White	Green	White/Green	(Hypothesis) χ^2
ABP1/ABP1	11	2297	1:208.82	
ABP1/abp1	802	2358	1:2.94	(1:3) 0.24*
ABP1/abp1, 35S::*ABP1*/–	54	815	1:15.09	(1:15) 0.002*
ABP1/abp1, pBAR1/–	264	788	1:2.98	(1:3) 0.005*

Transformed plants were selected by spraying with Liberty Herbicide. Siliques were opened to check color of immature seeds 8–12 days after flower opening. *ABP1/abp1,* 35S::*ABP1*/– represents plants heterozygous at the *abp1* locus and hemizygous for CaMV 35S::*ABP1*, *ABP1/abp1,* pBAR1/– represents plants heterozygous at the *abp1* locus and hemizygous for CaMV 35S vector only. Chi-square values are shown for the indicated segregation ratio.
*$P > 0.5$.

regated as expected for a single copy of the transgene per genome.

The developmental arrest in abp1 *embryo is at the early globular stage*

*abp1*mutants deviated from normal development only after the dermatogen stage (Fig. 3). Altered phenotypes became grossly apparent at the 32-cell stage. Newly formed cross walls of *abp1* embryos were misoriented (Fig. 3, cf. A with B).

With one important exception, morphogenesis during formation of the globular-staged embryo is driven predominantly by the placement of division planes rather than by selected cell elongation. That exception is the elongation of the single-celled zygote. However, after approximately the 32-cell dermatogen stage, cell elongation marks the acquisition of axiality and the embryo proper becomes bilaterally symmetrical (Mayer et al. 1993). This transition is manifest by the vertical elongation and subsequent anticlinal placement of division planes in the lower tier of cells as depicted at the top of Figure 3. The arrow in Figure 3A points to these elongated cells, and arrows labeled a in Figure 3 indicate the positions of the anticlinal walls that formed after vertical elongation. Lower tier cells do not elongate in the *abp1* embryos (Fig. 3, A vs. B) and the ordered flanks of cells that form by the coupling of elongation with anticlinal divisions at later developmental stages is also lacking (Fig. 3, cf. C with D, and E with F).

Cell division is aberrant in abp1 *mutants*

Not only is elongation and the anticlinal divisions needed to establish the vertical cell files, but formation of these lower tier cells via vertical divisions in the lower region of the early globular embryo is required also. This early step itself may be an auxin response that is disrupted by mislocated divisions in the *abp1* embryos.

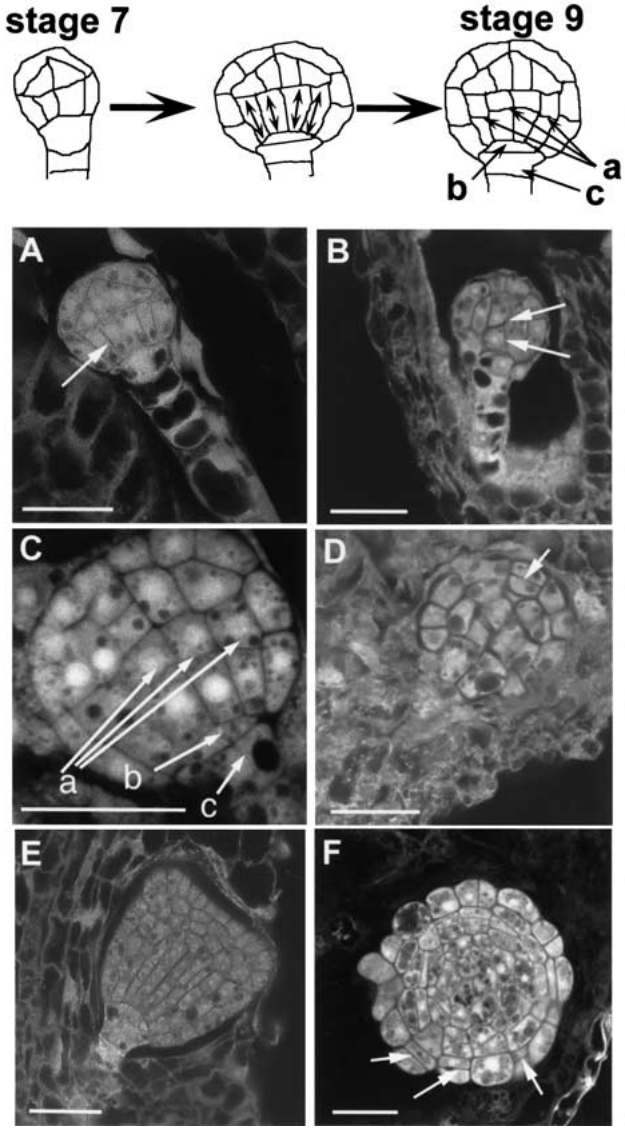

Figure 3. Development of *abp1* embryos. (*Top*) Normal progression of embryo development from dermatogen (stage 7) to midglobular (stage 9) embryos. Lower tier cells expand axially (indicated by double-headed arrows) before dividing anticlinally (arrows a). (*A–F*) Developmental stages are as follows: (*A*) wild-type embryo at early-globular stage (approximately stage 7). Arrow indicates elongated cells of the lower tier; (*B*) *abp1* embryo at early-globular stage, from the same silique as *A*. Arrows in *B* indicate the misoriented newly formed cross walls; (*C*) wild-type embryo at mid-globular stage (approximately stage 9). The arrows labeled a–c correspond to structures indicated at *top*; (*D*) *abp1* embryo at mid-globular stage, from the same silique as *C*. Arrow indicates a periclinal division in the outer layer cells; (*E*) wild-type embryo at early-heart stage; (*F*) *abp1* embryo arrested at globular stage, from the same silique as *E*. Arrows in *F* indicate periclinal divisions in the outer layer cells. The view shown in Fig. 3 is likely a medial transverse section. Images were obtained by confocal microscopy (YHS filter block, 568 nm excitation, Zeiss LSM 410 equipped with an Argon/Krypton laser). Fixation, clearing, and visualization of materials were by the method of Christensen et al. (1997). Scale bars, 25 μm.

abp1 embryos consistently displayed cell division abnormalities. Outer layer cells frequently divide periclinally (Fig. 3D,F). This effect on division may be an indirect effect due to a lack of polar growth or a direct effect of auxin mediated by *ABP1*. The later remains possible because it is known that auxin controls cell division. The boundary between the basal end of the *abp1* embryo proper and the suspensor was irregular (Fig. 3, cf. A with 3B; Fig. 4B–D). In normal development, the uppermost derivative of the basal cell becomes the hypophysis and divides asymmetrically, producing a lens-shaped cell (Fig. 3, arrow b) and a larger basal daughter cell (Fig. 3, arrow c), distal and proximal to the suspensor, respectively. These two derivatives of the hypophysis, which ultimately establish the embryonic root, were never observed in *abp1* mutant embryos (Figs. 3B and 4B–E), consistent with the general notion that root distal pattern-

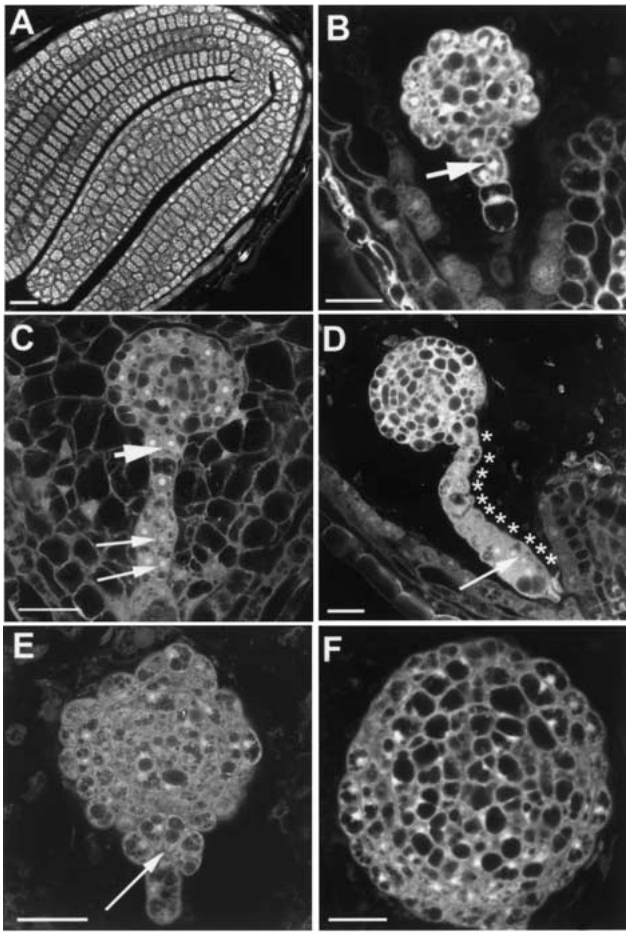

Figure 4. Developmental arrest of *abp1* embryos. (*A*) Abp1 $^{+}$ embryo at mature cotyledon stage, (*B–F*) Abp1^{-} embryos, from the same silique as *A*. Arrows with large arrowhead in *B* and *C* indicate an extra cell division in the apical suspensor. Long arrows in *C* indicate periclinal cell division. Arrow in *D* indicates an anticlinal cell division. Asterisks indicate individual cells along the suspensor. Arrow in *E* indicates the abnormal junction between embryo proper and suspensor. Scale bars, 25 μm.

ing is established through auxin (Sabatini et al. 1999). *abp1* mutant embryos lack the characteristic cell arrangement and shape that represent the incipient shoot and root anlagen observed in transition-stage embryos (Fig. 4B) and, as well, lack the internal tissue organization of later stages (Fig. 4, cf. A with B–F). Finally, as seen in Figure 4, B, D, and F, *abp1* cells are less elongated (average axial ratios = 1.4 vs. axial ratio of wild-type cotyledon cells = 2.2, Fig. 4A).

In contrast to normal wild-type embryogenesis, the suspensor apparatus of *abp1* mutants failed to degenerate, indicating that normal embryo progression must signal the onset of programmed cell death in the suspensor. In the auxin mutant, *bodenlos* (Hammann et al. 1999), the apical cell of the two-cell embryo divides vertically instead of anticlinally, thus failing to form the basal root initial. *abp1* mutants consistently showed a similar aberrant division, although in the apical suspensor (Fig. 4B,C), giving rise to twinned cells that continue to divide to form a cluster (Fig. 4C,D, long arrow). Such an aberrant division in the apical suspensor occurs in the auxin-insensitive mutant, *axr6* (Hobbie et al. 2000), although perhaps at an earlier stage. The suspensors of *axr6* and *abp1* also share ectopic periclinal divisions (Fig. 4C, long arrows), but *abp1* mutants undergo additional ectopic anticlinal divisions to produce suspensors that are longer (Fig. 4D, asterisks) than the typical 6- to 9-cell file. At a later stage, the abnormal junction between *abp1* embryo proper and the suspensor became much more pronounced (Fig. 4E).

BY-2 cells lacking detectable ABP1 *proliferate but lack auxin-inducible cell elongation*

Because *abp1* mutant embryos proliferate into large masses of cells, the defect does not appear to affect a cell-vital function, but rather disrupts the organization of *Arabidopsis* embryos established by organized cell divisions and elongations. Cell lines are the choice system to precisely examine mechanisms controlling cell division and elongation. Because it is not possible to generate cell lines from *Arabidopsis* embryos in the early globular stage (Luo and Koop 1997), the stage at which *abp1* embryos are blocked, we have chosen to reduce *ABP1* expression in an established cell line designated BY-2. An additional advantage of testing the role of *ABP1* in BY-2 cells is that cell division and elongation has already been characterized in this line (Hasezawa and Syono 1983). The large number of smaller cells in *abp1* embryos also suggests that cell expansion is affected to a greater degree than cell division, aside from the aberrant division planes discussed above. To test this further, antisense suppression of *ABP1* was used to generate a BY-2 cell line (designated NAS1) with a level of ABP1 protein that was undetectable by three different antibodies (Fig. 5A). NAS1 cells proliferated for more than 1 yr, although at a lower rate of fresh weight increase compared with the control line (Fig. 5B). The fresh weight increase in control cells that occurs between 3 and 6 d after transfer is due primarily to cell division with regenerative expansion, whereas the fresh weight increases after 7 d is due primarily to net cell expansion without cell division (data not shown). At the phase of maximum cell division (days 4–5), the rate of NAS1 cell division was essentially normal (Fig. 5B), although cells grew as clumps of smaller cells, unlike the linear growth of control cells (Fig. 5, cf. C and D). In contrast to division, little increase in fresh weight was observed for NAS1 cells during the expansion phase (after day 7). NAS1 cells were substantially shorter (control length = 131 ± 41 μm vs. NAS1 = 65 ± 11 μm) and smaller (control area = 2666 ± 343 μm^2 vs. NAS1 = 1836 ± 231 μm^2) than the control lines (Fig. 5, cf. E with F).

The *ABP1* antisensed line lacks auxin-induced cell elongation (Fig. 6). Specifically, whereas expansion to recharge cell volume after division is present (meristematic growth), auxin-induced elongation that increases cell volume beyond the dividing cell (vegetative growth) is completely absent. Auxin-induced elongation was observed in control cells by transferring 7-day-old cells to the indicated low auxin concentrations, but similarly treated NAS1 cells were completely unresponsive to auxin. The loss of auxin-inducible elongation was shown with the synthetic auxin, 1-NAA.

Higher concentrations of auxin induced cell division and DNA synthesis, and this response was measured in the NAS1 cells. To test this result more precisely, cells were synchronized by auxin starvation for 3 d (Fig. 7A). Under these conditions, NAS1 cells were significantly shorter (control length = 324 ± 42 μm vs. NAS1 = 182 ± 22 μm) and smaller (control area = 22,664 ± 2412 μm^2 vs. NAS1 = 13,668 ± 1644 μm^2) than control cells (Fig. 7B,E,F) as was already shown in nonsynchronous cells (Fig. 5). Note that the auxin-induced elongation effect in control cells becomes obscured by the 3-day starvation step needed to synchronize these cells. Auxin-induced cell division in NAS1 was reduced in NAS1 compared with control cells (Fig. 7D); however, DNA synthesis as measured by [^{3}H]thymidine was nearly the same on a per cell basis (Fig. 7, cf. C with D). Interestingly, not only was elongation reduced in NAS1 cells, but it also grew as a globular mass in culture, reminiscent of embryonic cells in the *abp1* mutant (Fig. 4F).

Discussion

Plant embryogenesis involves a strict orchestration of cell division, elongation, and differentiation (Meinke 1995; Laux and Jürgens 1997). A current hypothesis is that the plant hormone auxin provides positional information interpreted by the developing embryo to direct which cells divide and which cells elongate to form this polarized structure (Vroemen et al. 1999).

A regulatory role for auxin in embryogenic patterning has already been established by multiple approaches. For example, manipulation of the amount and spatial distribution of auxin (Liu et al. 1993; Fischer and Neuhaus 1996; Hadfi et al. 1998) in developing zygotic embryos phenocopies development observed in a number of embryo defective mutants (Mayer et al. 1991). Many em-

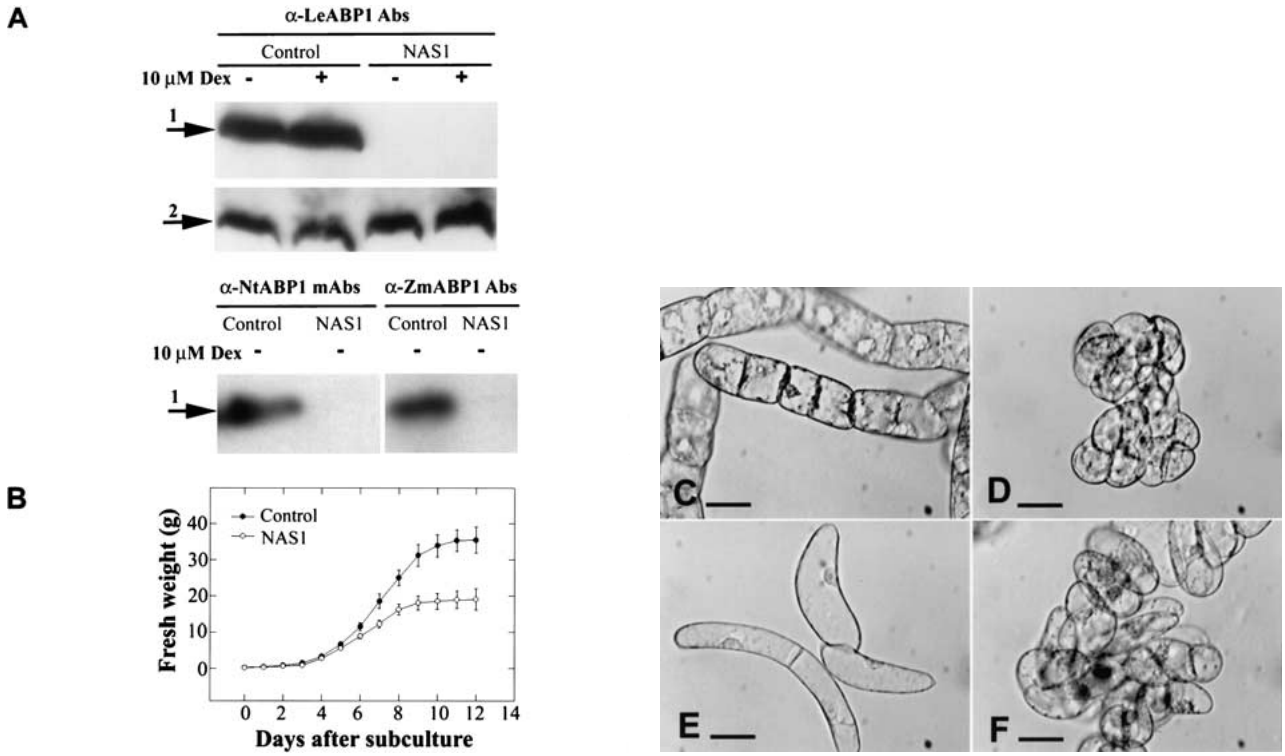

Figure 5. Antisense suppression of *ABP1* in BY-2 cells. (*A*) Immunoblot analyses of ABP1 protein levels in crude microsomal fractions from control and an *ABP1*-antisense suppressed BY-2 cell line (designated NAS1) 5 d after subculture in the presence or absence of 10 μM dexamethasone (Dex), using anti-tomato ABP1 polyclonal Abs (α-LeABP1 Abs, *top*), anti-tobacco ABP1 monoclonal antibodies (α-NtABP1 mAbs, *bottom*, *left*), and anti-maize ABP1 polyclonal antibodiess (α-ZmABP1 Abs, *bottom*, *right*). Arrow 1 indicates the expected band for NtABP1; arrow 2 indicates a nonspecific band recognized by anti-LeABP1 Abs, which serves as an internal loading control. Even though the antisense construct is driven by a Dex-inducible promoter, these cells essentially show constitutive suppression of ABP1 due to high basal level of expression of the antisense construct. Therefore, further analyses shown were done in the absence of Dex. (*B*) Growth rate of NAS1 cells. Medium containing 2,4-D (9×10^{-7} M) were seeded with equal numbers of cells (1.7×10^6 cells) and fresh weight determined over time. Fresh weight increase after day 7 is due primarily to cell expansion with little accompanying division. The graph shows the mean ±S.E. of three replicates. (*C*) Control and (*D*) NAS1 cells in the division phase, 4 d after subculture. (*E*) Control and (*F*) NAS1 cells in the expansion stage, 11 d after subculture. Scale bars, 50 μm.

bryo-patterning mutants have altered auxin sensitivities or are associated with auxin signal transduction (Hardtke and Berleth 1998; Hammann et al. 1999; Hobbie et al. 2000) beginning as early as the primary zygotic division. Unlike the established role for auxin in embryogenesis, the receptor(s) mediating any auxin action is unclear. This study provides direct evidence that ABP1 has a role in embryonic morphogenesis.

The transition from early globular embryos to heart-stage embryos marks the acquisition of axiality. This involves the longitudinal expansion of a specific set of cells at a determined time in development. In principle, the failure of these lower tier cells in *abp1* embryos to elongate could be interpreted in the following three ways: ABP1 mediates auxin-induced cell elongation, ABP1 may be important in establishing embryo polarity, ABP1 may establish individual cell polarity. A failure in either organ or cell polarity may mean that cells are able to undergo an auxin-induced elongation but fail to do so because they are unable to direct the axis of expansion. The results shown here by use of cells in culture preclude a direct role for ABP1 in embryo polarity as a requisite for directional outgrowth of embryonic cells, because single cells in culture that have reduced levels of ABP1 exhibit the same defect in cell elongation as observed in *Arabidopsis abp1* embryos. This precludes a requirement for established organ polarity in ABP1-mediated cell elongation. However, the possibility that ABP1 establishes cell polarity that may serve as a requisite for auxin-induced expansion is not excluded by our data. On the basis of morphological markers, polarity seems to be present in *abp1* cells from the onset of embryogenesis, because the zygote polarly divides and correctly places division planes up to the early embryogenesis stage. Moreover, neither *abp1* embryo cells nor NAS1 cells (*ABP1* suppressed, Fig. 7F) are isodiametric, suggesting that cell polarity is present in the absence of ABP1. This is the case for *abp1* embryonic cells as well because many cells have an axial ratio of 1.4. Thus, whereas a role for establishing cell polarity cannot be excluded, the evidence favors the conclusion that ABP1 mediates auxin-induced elongation.

Two decades of biochemical research has brought us to the conclusion that ABP1 binds auxins specifically, and

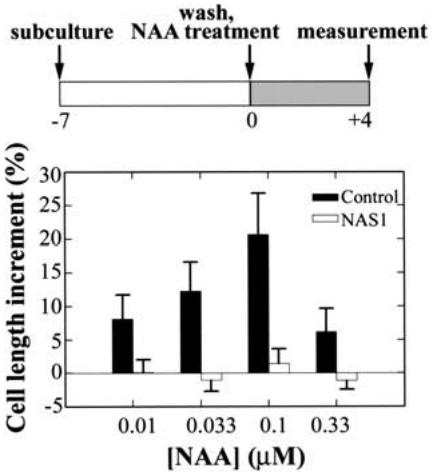

Figure 6. Auxin-induced cell elongation in BY-2 cells. At *top* is shown the experimental scheme in which numbers indicate days. The lengths of control (solid bars) and NAS1 (open bars) cells cultured in the indicated concentrations of NAA were measured. Cell length increment is shown as a percentage over growth in the absence of auxin. The graph shows the mean ±S.E. of three replicates.

the binding affinities are consistent with a physiological role (Ray et al. 1977; Jones 1994; Napier 1995). Thus, as an auxin-binding protein, ABP1 could be an auxin receptor or a protein that modulates the concentration of auxin. In either case, auxin binding to ABP1 could lead to an identifiable auxin response, directly in the first case, and indirectly in the second. Both overexpression of ABP1 (Jones et al. 1998) and loss of function (this work), conceivably could have effects that manifest as a hormone response. However, it has been shown that the concentration of ABP1, on the basis of auxin-binding measurements, is in the range of 0.1–10 picomoles per gram fresh weight (Jones et al. 1984; Harnden and Jones 1995; Shimomura et al. 1999), whereas the presumed operating concentration of auxin is 1000-fold higher. Therefore, even 100% occupancy of ABP1 would have a negligible effect of free IAA levels, unless assumptions are made about the microenvironment concentrations of auxin and ABP1. Finally, it is difficult to reconcile the observed acquisition of an auxin-dependent response in ABP1-overexpressing cells if ABP1 functioned as an auxin-sequestrating protein. The expected effect of overexpressing a hormone-sequestrating protein is the decrease in hormone sensitivity, not the observed conferral of an ectopic auxin-dependent response (Jones et al. 1998). In summary, the possibility that ABP1 controls auxin economy or concentration is questionable and, therefore, the observed acquisition of an auxin response in the *ABP1*-overexpressing cells (Jones et al. 1998), and the loss in a null mutant (this work), respectively, is supportive of a receptor function rather than a sequestering function.

The uniqueness of ABP1 provides both an obstacle and an opportunity. Its lack of sequence identity to any other eukaryotic protein annotated in databases at this time,

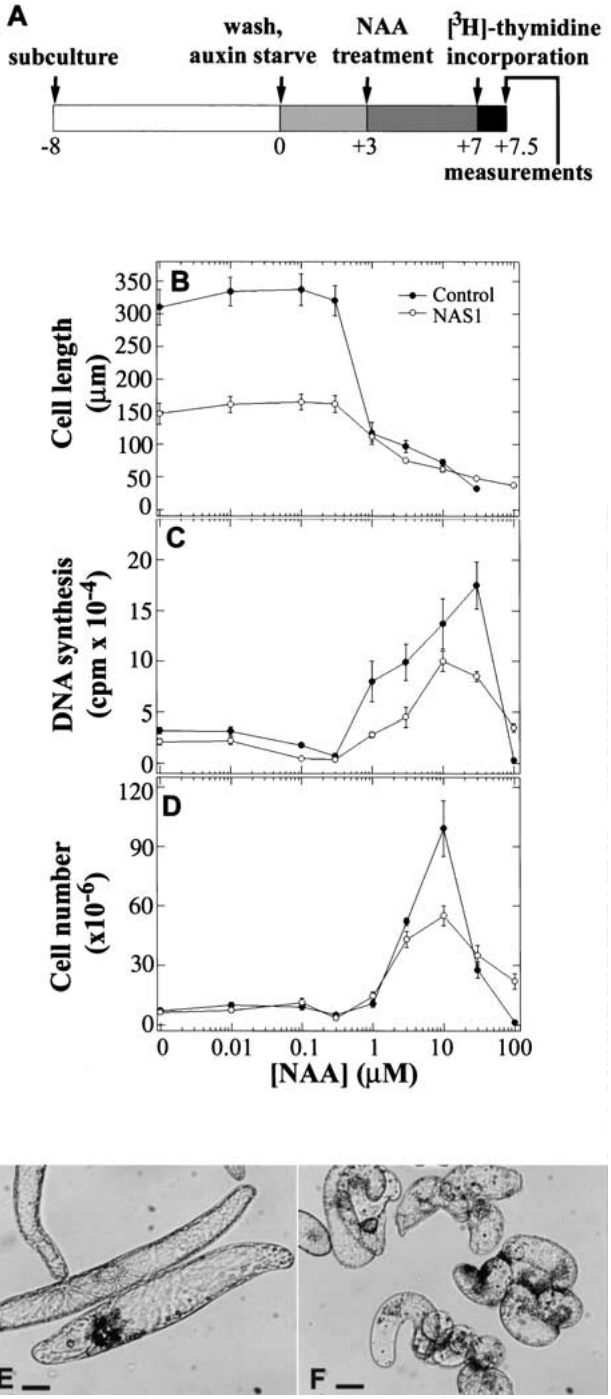

Figure 7. Dose response of *ABP1* antisense suppression in BY-2 cells (NAS1) to auxin. (*A*) The experimental scheme. Numbers indicate the time course (day). Cells were synchronized by auxin starvation for 3 d, then transferred to medium containing the indicated concentrations of auxin. A pulse of [^{3}H] thymidine was applied at day 7. Cell length, [^{3}H]thymidine incorporation and length were determined 12 h later. (*B–D*) Measurements of the response of control and NAS1 cells to NAA in terms of cell length (*B*), DNA synthesis by [^{3}H]thymidine incorporation (*C*), and cell number (*D*). Starting cultures were seeded with 4.0×10^6 cells. The graphs show the mean ±S.E. of three replicates. (*E,F*) Control and NAS1 cells at 10^{-7} M of NAA. Images taken 4.5 d after NAA treatment. Scale bars, 50 μm.

much less to a well understood animal hormone receptor, does not yield any clues on mode of action. On the other hand, its novelty opens up a new signal transduction pathway. Whereas ABP1 meets the two criteria firmly established for hormone receptor function (Birnbaumer et al. 1974), namely, it binds the hormone and the auxin/ABP1 complex evokes a so-called hormone response, ABP1 may not function within a classical hormone-transduction mode.

Receptor function defined broadly includes the possibility of ligand-regulated enzyme activity, and in this instance, we must consider possibilities outside of the realm of well-studied receptor-enzyme paradigms such as receptor kinases. For example, it is possible that ABP1 has some ligand-regulated chaperone role. ABP1 is found predominantly in the lumen of the endoplasmic reticulum, although it is certainly at detectable levels throughout the endomembrane system (Jones and Herman 1993; Henderson et al. 1997) and at the plasma membrane/cell wall interface (Deikman et al. 1995). Our working hypothesis is that ABP1 regulates the direction or flux of wall materials. Because all cell wall precursors, except cellulose, pass near ABP1, it is not unreasonable to speculate that some growth-limiting component interacts with ABP1 in an auxin-dependent manner. This interaction could affect either how much of this precursor passes or the pathway it takes. As plant cell division also involves the movement of cell wall precursors, albeit along the phragmoplast rather than the TGN, ABP1 conceivably controls division as well. This possibility is consistent with the results shown in the present study. The results here are also consistent with a model whereby ABP1 interacts with a docking protein at the plasma membrane (Klämbt 1990) and our working hypothesis includes this variation on the speculated mode of action.

The shown results here do not preclude the involvement of multiple auxin receptors in expansion and division, nor does the existence of a single *ABP1* gene in *Arabidopsis* indicate that all auxin responses are mediated through ABP1. It has been shown repeatedly that auxin responses can be dissected into biochemically and spatially separable components (Vanderhoef and Dute 1981, and references therein). For example, cell expansion can be uncoupled from auxin-induced gene expression, therefore, there is no need to mandate that an ER-localized auxin receptor mediates auxin-regulated gene expression. Conversely, the rapid effects at the plasma membrane induced by auxin need not be directly mediated by a nuclear-localized auxin receptor. Multiple auxin receptors controlling a single auxin response are possible and supported by previous studies.

The phenotype of the Abp1$^-$ embryos and the NAS1 cells address a long-standing question about cell growth modes: Is meristematic growth mechanistically the same as cell growth involved in tissue growth? The results here show that cell volume recharge occurring in daughter cells following division (i.e., a component of meristematic growth) is distinct mechanistically from the cell volume increase occurring in most tissue growth and tropisms (expansion growth). Clearly, ABP1 is not essential for cell volume recharge involved in meristematic growth.

In summary, the data presented here taken from loss-of-function phenotypes in both whole plant and cells in culture indicate that ABP1 mediates auxin-regulated cell elongation and cell division either directly or indirectly. The data are consistent with the conclusion drawn from the gain-of-function study (Jones et al. 1998). ABP1 mediation of cell elongation is probably not restricted to the young embryo as indicated by the null mutant because ABP1 is found in other rapidly growing zones of the plant. Tests for ABP1 involvement have suggested that it functions at many points during plant growth and development. The challenge ahead includes finding weak or conditional alleles of *abp1* to ascertain and delimit the role of the gene in auxin signal transduction.

Materials and methods

ABP1 *T-DNA insertion allele screening by PCR*

A total of 60,480 T-DNA-tagged *Arabidopsis* generated at the University of Wisconsin Knockout *Arabidopsis* facility (www.biotech.wisc.edu/Arabidopsis) were screened for *ABP1* insertion alleles by PCR. Primers specific for the T-DNA left-border (5′-CATTTTATAATAACGCTGCGGACATCTAC-3′) and the right-border (5′-TCCCAACAGTTGCGCACCT GAATG-3′) were used in tandem with *ABP1*-specific primers (forward, 5′-ACGAGAAAATCATACCAATTCGGACTAACC-3′ and reverse, 5′-GTATCTACGTAGTGTCACAAAACCTCA AC-3′) for identification of mutant lines.

ABP1 *T-DNA insertion allele screening by Southern blot*

Ten micrograms of genomic DNA from wild-type and mutant plants were digested with *Xho*I, *Xba*I, and *Hin*dIII, respectively, run on a 0.7% agarose gel, and transferred to nylon membrane. The blots were hybridized with a genomic *ABP1* probe generated by use of *ABP1* forward and reverse primers, a cDNA PCR product (whole coding region of *ABP1* gene), and a T-DNA pD991 plasmid DNA (~5.5 kb). Blots were washed under high [2× SSC, 0.1% (w/v) SDS, twice for 15 min at 65°C; 0.1× SSC and 0.1% SDS twice for 15 min at 65°C] and low (5× SSC, 0.1% SDS, twice for 15 min at 42°C; 1× SSC and 0.1% SDS, once for 15 min at 42°C) stringency conditions, respectively.

Genetic complementation

The full-length *Arabidopsis ABP1* cDNA coding region was subcloned into binary vector pBAR1 (35S promoter), derived from pGPTV-BAR (delta), by use of restriction sites *Xho*I and *Xba*I and confirmed by sequencing. Binary vectors pBAR1 35S::ABP1 and vector-only plasmid (pBAR1) were used to transform mutant plants heterozygous at the *abp1* locus by use of standard *Agrobacterium*-mediated vacuum infiltration transformation (Bechtold and Pelletier 1998). T1 plants were selected by spraying with 1:10,000 dilution of Liberty Herbicide (AgrEvo) plus 0.005% Silwet. PCR methods were used to distinguish mutant plants heterozygous at the *abp1* locus from wild-type plants in T1-transformed populations. Plants heterozygous at the *abp1* locus and hemizygous for 35S:ABP1 were used to analyze immature seed segregation within siliques. Plants hetero-

zygous at the *abp1* locus that were transformed with pBAR1 vector-only were used as control.

Antisense suppression of ABP1 in a BY-2 cell line and characterization

The full-length tobacco *ABP1* cDNA coding region was cloned into binary vector pTA7002 (kindly provided by Dr. Nam-Hai Chua, Rockefeller University) to form pNAS1 and confirmed by sequencing. Tobacco BY-2 cells were transformed by *Agrobacterium*-mediated transformation. Approximately 30 hygromycin-resistant colonies were pooled, collectively designated NAS1, and maintained in the same way as control BY-2 cells. The ABP1 protein expression level in wild-type and transformed lines were measured by immunoblot analysis by use of a polyclonal antiserum directed against recombinant tomato ABP1 (lacking the signal sequence), and confirmed by anti-tobacco ABP1 monoclonal antibodies (kindly provided by Dr. Catherine Perrot-Rechenmann, Institut des Sciences Végétales, CNRS, France) and anti-native maize ABP1 polyclonal antibodies (kindly provided by Dr. Richard M. Napier, Horticulture Research International, UK). In the 1-NAA treatment, cells (7 d after subculture) were washed with hormone-free medium and treatment with a different concentration of NAA. Cell length was measured 4 d after NAA treatment. In the experiment on cell division and thymidine incorporation, after 8 d in culture, cells were washed with hormone-free medium and auxin-starved for 3 d. The cells were then treated with NAA at a different concentration. Cell length and cell area were measured digitally from at least 100 cells.

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