Title
Embryonic lethality of Arabidopsis abp1-1 is caused by deletion of the adjacent BSM gene

Authors
Dai, Xinhua
Zhang, Yi
Zhang, Da
et al.

Embryonic lethality of *Arabidopsis abp1-1* is caused by deletion of the adjacent *BSM* gene

Xinhua Dai[1], **Yi Zhang**[1,2], **Da Zhang**[1,3], **Jilin Chen**[1,4], **Xiuhua Gao**[1,5], **Mark Estelle**[1,2], and **Yunde Zhao**[1,*]

[1]Section of Cell and Developmental Biology, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093-0116, USA

[2]Howard Hughes Medical Institute, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093-0116, USA

[3]College of Life Sciences, Northeast Agricultural University, Harbin 150030, China

[4]College of Life Sciences, Xiamen University, Xiang An Nan Road, Xiamen 361102, Fujian Province, China

[5]Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, 1 West Beichan Road, Beijing 100101, China

Abstract

Decades of research have suggested that AUXIN BINDING PROTEIN 1 (ABP1) is an essential membrane-associated auxin receptor, but recent findings directly contradict this view. Here we show that embryonic lethality observed in *abp1-1*, which has been a cornerstone of ABP1 studies, is caused by the deletion of the neighbouring *BELAYA SMERT* (*BSM*) gene, not by disruption of *ABP1*. On the basis of our results, we conclude that ABP1 is not essential for *Arabidopsis* development.

For decades, AUXIN BINDING PROTEIN 1 (ABP1) has been described as a candidate plasma membrane-associated auxin receptor[1,2]. ABP1 was first identified on the basis of its ability to bind auxin *in vitro*[1,3]. Early biochemical and structural studies clearly demonstrated that ABP1 could bind auxin. It was later proposed that ABP1 plays an essential role in *Arabidopsis* development when the first T-DNA insertional mutation in *ABP1* (*abp1-1*) was reported to cause embryonic lethality[4]. Because the knockout of *ABP1* in *abp1-1* appeared to result in embryonic lethality, subsequent efforts focused on isolating weak alleles of *abp1* and developing knockdown lines of *ABP1* (refs [5,6]). The results of

these studies suggested that ABP1 is involved in almost every aspect of plant growth and development[5–11]. However, the roles of ABP1 in auxin signalling and *Arabidopsis* development were called into question when we described two new *abp1* mutants[12]. Our *abp1-c1* and *abp1-TD1* mutations are null alleles, but the mutants are indistinguishable from wild-type (WT) plants, demonstrating that ABP1 is not required for auxin signalling or *Arabidopsis* development under normal growth conditions[12]. It has been difficult for auxin biologists to reconcile these contradictory results. On the one hand, studies from multiple laboratories appear to support the important roles for ABP1 in auxin signalling and plant development. On the other hand, our new *abp1* alleles, which were null alleles based on well-accepted criteria, lacked obvious developmental defects[12]. Recently, it was suggested that ABP1-related genetic materials be exchanged and re-analysed independently by different laboratories[13–15]. The recent publication of the whole genome sequence of *abp1-5* is one step forward in efforts to clarify the ABP1 field[16]. It was revealed that *abp1-5* contains more than 8,000 mutations/single nucleotide polymorphisms, and that mutations in the *Phytochrome B* (*PHYB*) gene are probably responsible for some defects previously ascribed to *abp1-5* (ref. 16).

In this paper, we re-analyse the *abp1-1* mutant, which has been instrumental in assigning an essential role for ABP1 as an auxin receptor[4]. The initial report demonstrated that *abp1-1* was embryo lethal and that expression of *ABP1* complementary DNA (cDNA) using the *CaMV 35S* promoter had rescued the embryo-lethal phenotype[4]. The complementation result appeared to be reproducible when it was reported last year that overexpression of WT *ABP1* cDNA using the *CaMV 35S* promoter had rescued the *abp1-1* embryo-lethal phenotype[17]. However, earlier this year, it was reported that *abp1-1* could not be rescued by either *ABP1* cDNA or an *ABP1* genomic fragment using the *CaMV 35S* promoter or the *ABP1* native promoter[18]. Therefore, it has not been clearly demonstrated that disruption of *ABP1* in the *abp1-1* mutant is responsible for the embryo-lethal phenotype.

Because of these contradictory reports and the lack of developmental defects in our *abp1* null mutants, we and others[15] hypothesized that the embryo lethal phenotype in *abp1-1* might not be caused by the disruption of *ABP1*. Habets & Offringa[15] went further to suggest that the embryo lethal phenotype might be caused by disrupting the neighbouring gene, *At4g02990* (*BSM*). Our re-analysis of the Southern blot results using *ABP1* as probes suggests that the transfer DNA (T-DNA) insertion in *abp1-1* is likely to be complex[4]. For example, DNA from WT plants is predicted to generate a 6.4 kb XhoI fragment, which was observed as predicted[4]. Three XhoI fragments (6.4 kb, 7.5 kb and 1.8 kb) from the *abp1*^{+/−} plants were expected, but only the 6.4 kb fragment (WT) and a 5.5 kb fragment (mutant) were detected[4], suggesting that part of the flanking DNA at the T-DNA insertion site was deleted or rearranged. We also note that the defects in embryogenesis observed in *abp1-1* closely resemble those observed in the *bsm/at4g02990* mutant[19], which is located directly adjacent to *ABP1* with overlapping promoters (Fig. 1a). Therefore, we hypothesized that the T-DNA insertion in *abp1-1* might also disrupt *At4g02990* in addition to compromising *ABP1*. To test this idea, we introduced a WT copy of *At4g02990* into *abp1-1*^{+/−} plants to determine whether *At4g02990* can rescue the embryo-lethal phenotype. We decided not to use the native *At4g02990* promoter for two reasons. First, the fact that *ABP1* and *At4g02990* promoters overlap but with opposite orientation (Fig. 1a) may complicate the

interpretation of the results. Second, *At4g02990* does not contain any introns, which renders it more difficult to differentiate the transgene from the native *At4g02990* if the native promoter and untranslated regions are included in the complementation construct. We transformed a population of plants segregating for *abp1-1*^{+/−} and WT plants with the *35S:At4g02990* construct and selected transgenic plants on medium containing both kanamycin and hygromycin, selecting for the *abp1-1* T-DNA insertion and the transgene, respectively. Among the 18 double-resistant plants we obtained, two were *abp1-1* homozygous (Fig. 1b), demonstrating that introduction of *At4g02990* into *abp1-1* completely rescued the embryo-lethal phenotype. We further analysed the progenies from the two *abp1-1* homozygous plants and found that all of the T2 plants were *abp1-1* homozygous (data not shown), demonstrating that the rescued lines can be stably transmitted to the next generations. We also genotyped 447 T2 plants from a single T1 plant that had the *abp1*^{+/−} genotype with the *35S:At4g02990* transgene. We found that 13% (58/447) was *abp1-1* homozygous, 58% (260/447) was *abp1-1* heterozygous and 29% (129/447) WT, further supporting our conclusion that the embryo-lethal phenotype in *abp1-1* was caused by disrupting *BSM/At4g02990*, not by inactivating *ABP1*. At this point, it is not clear why the *ABP1* cDNA appeared to rescue the *abp1-1* embryo phenotype in the original report[4].

The availability of *abp1-1* homozygous plants rescued by the *35S: At4g02990* transgene (referred as *abp1-1*^{−/−} hereafter) provided us with an opportunity to analyse the nature of the T-DNA insertion in *abp1-1*. The T-DNA insertion was easily genotyped using polymerase chain reaction (PCR)-based methods[20] (Fig. 1b). We resequenced the flanking DNA and found that the T-DNA was inserted 51 bp downstream of the ATG start codon, a result that was the same as previously reported[4]. We tried to define the left border junction, but failed to amplify the flanking DNA using various combinations of T-DNA-specific and gene-specific primers. We hypothesized that part of the genomic DNA including *At4g02990* might have been deleted or rearranged. We found that several pairs of primers that successfully amplified DNA fragments with the predicted sizes from WT DNA or *abp1*^{+/−} samples failed to amplify a fragment from DNA samples of the *abp1-1*^{−/−} plants (Fig. 1c), suggesting that *At4g02990* has been disrupted by the T-DNA insertion in *abp1-1*^{−/−} (Fig. 1d).

We next determined whether *abp1-1*^{−/−} is a null allele or not. We first used PCR with reverse transcription (RT–PCR) to determine whether *ABP1* is expressed in *abp1-1*^{−/−}. As shown in Fig. 2a, *abp1-1*^{−/−} plants did not produce full-length *ABP1* messenger RNA. We next investigated whether partial *ABP1* mRNA was still produced in *abp1-1*^{−/−} plants by using two primers downstream of the T-DNA insertion site (Fig. 2a). Interestingly, the primer pair amplified similar bands from both WT and the *abp1-1*^{−/−} samples, suggesting that *abp1-1*^{−/−} still produced truncated *ABP1* mRNA (Fig. 2a). The truncated *ABP1* mRNA can potentially produce partial ABP1 proteins that lack the first 35 amino acid residues of the full-length ABP1 proteins. We then performed a western blot to determine whether the truncated *ABP1* mRNA was actually translated into partial ABP1 proteins. As shown in Fig. 2b, we did not detect either full-length ABP1 protein or truncated ABP1 protein in the *abp1-1*^{−/−} sample. A caveat of the western blot result is that we could not rule out the possibility that the antibody may not recognize truncated ABP1 proteins if the antigenic epitope is located at the amino-

terminal region of ABP1. Nevertheless, the homozygous $abp1$-$1^{-/-}$ plants were viable and grew to full maturity (Fig. 2c).

We have clearly demonstrated that the previously reported embryonic lethality in *abp1-1* was caused by the deletion of *BSM/At4g02990*, and not by the disruption of *ABP1* (Figs 1 and 2). Together with our recently published new *abp1* alleles[12], we conclude that ABP1 is not essential and does not appear to have a major role in *Arabidopsis* growth and development.

Materials and methods

Plant materials and genotyping information

The *abp1-1* (stock no. CS6498) was obtained from the *Arabidopsis* Stock Center. To genotype *abp1-1*, we used A1, A3 and the T-DNA specific primer PD991-RB. The primer sequences are listed in Supplementary Table I.

Complementation of *abp1-1* by *35S:At4g02990*

The coding region of *At4g02990* was amplified by PCR using the primer pair (5′-CATTTCATTTGGAGAGGACACGATGAAGATTAGGTTCT GTAATGGCT-3′ and 5′-CTGGGAGGCCTGGATCGATGGTTT ATGCAAACTCCTCGTC-3′). The open reading frame was then put under the control of the CaMV35S promoter. The *35S:At4g02990* cassette was then cloned into the vector pHDE. The complementation construct was transformed into *abp1-1* heterozygous plants by floral dipping. Transgenic plants were selected on 25 μg ml^{-1} kanamycin and 16 μg ml^{-1} hygromycin.

Western blot and RT–PCR

Western blot was conducted as previously described[12]. To determine whether *abp1-1*$^{-/-}$ still produced *ABP1* mRNA, we conducted RT–PCR analysis. For each genotype, five biological replicates were prepared. Total RNAs from samples were extracted using the Qiagen Rneasy Plant Mini Kit (cat. no. 74904) following the manufacturer's instructions. RNA samples were then treated with DNase and purified before used for RT–PCR analysis. The RT–PCR products were amplified with 45 saturated PCR cycles and loaded on to 1.2% agarose gel. The housekeeping gene *PP2A* was used as a control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Jones A. AUXIN-BINDING PROTEINS. Annu Rev Plant Physiol Plant Mol Biol. 1994; 45:393–420.

2. Xu T, et al. Cell surface ABP1-TMK auxin-sensing complex activates ROP GTPase signaling. Science. 2014; 343:1025–1028. [PubMed: 24578577]

3. Woo EJ, et al. Crystal structure of auxin-binding protein 1 in complex with auxin. EMBO J. 2002; 21:2877–2885. [PubMed: 12065401]

4. Chen JG, Ullah H, Young JC, Sussman MR, Jones AM. ABP1 is required for organized cell elongation and division in Arabidopsis embryogenesis. Genes Dev. 2001; 15:902–911. [PubMed: 11297513]

5. David KM, et al. The auxin-binding protein 1 is essential for the control of cell cycle. Plant J. 2007; 50:197–206. [PubMed: 17376160]

6. Braun N, et al. Conditional repression of AUXIN BINDING PROTEIN1 reveals that it coordinates cell division and cell expansion during postembryonic shoot development in Arabidopsis and tobacco. Plant Cell. 2008; 20:2746–2762. [PubMed: 18952781]

7. Tromas A, et al. The AUXIN BINDING PROTEIN 1 is required for differential auxin responses mediating root growth. PLoS ONE. 2009; 4:e6648. [PubMed: 19777056]

8. Robert S, et al. ABP1 mediates auxin inhibition of clathrin-dependent endocytosis in Arabidopsis. Cell. 2010; 143:111–121. [PubMed: 20887896]

9. Xu T, et al. Cell surface- and rho GTPase-based auxin signaling controls cellular interdigitation in Arabidopsis. Cell. 2010; 143:99–110. [PubMed: 20887895]

10. Tromas A, et al. Auxin-binding protein 1 is a negative regulator of the SCF (TIR1/AFB) pathway. Nature Commun. 2013; 4:2496. [PubMed: 24051655]

11. Chen X, et al. Inhibition of cell expansion by rapid ABP1-mediated auxin effect on microtubules. Nature. 2014; 516:90–93. [PubMed: 25409144]

12. Gao Y, et al. Auxin binding protein 1 (ABP1) is not required for either auxin signaling or Arabidopsis development. Proc Natl Acad Sci USA. 2015; 112:2275–2280. [PubMed: 25646447]

13. Tena G. Auxin signalling: ABP1 springs a surprise. Nature Plants. 2015; 1:15028.

14. Liu CM. Auxin binding protein 1 (ABP1): a matter of fact. J Integr Plant Biol. 2015; 57:234–235. [PubMed: 25664934]

15. Habets ME, Offringa R. Auxin binding protein 1: a red herring after all? Mol Plant. 2015; 8:1131–1134. [PubMed: 25917757]

16. Enders TA, Oh S, Yang Z, Montgomery BL, Strader LC. Genome sequencing of Arabidopsis abp1-5 reveals second-site mutations that may affect phenotypes. Plant Cell. 2015; 27:1820–1826. [PubMed: 26106149]

17. Effendi Y, Ferro N, Labusch C, Geisler M, Scherer GF. Complementation of the embryo-lethal T-DNA insertion mutant of AUXIN-BINDING-PROTEIN 1 (ABP1) with abp1 point mutated versions reveals crosstalk of ABP1 and phytochromes. J Exp Bot. 2015; 66:403–418. [PubMed: 25392478]

18. Grones P, et al. Auxin-binding pocket of ABP1 is crucial for its gain-of-function cellular and developmental roles. J Exp Bot. 2015; 66:5055–5065. [PubMed: 25922490]

19. Babiychuk E, et al. Plastid gene expression and plant development require a plastidic protein of the mitochondrial transcription termination factor family. Proc Natl Acad Sci USA. 2011; 108:6674–6679. [PubMed: 21464319]

20. Alonso JM, et al. Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science. 2003; 301:653–657. [PubMed: 12893945]
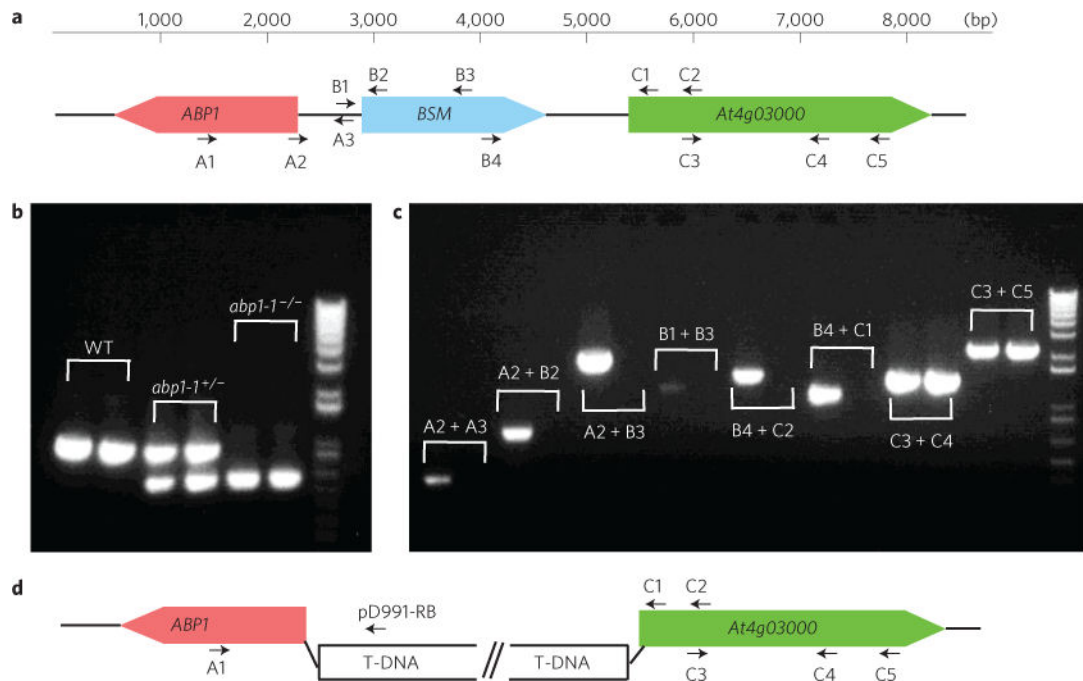
Figure 1. Rescue *abp1-1* by the *35S:At4g02990/BSM* transgene

a, A schematic description of the structures of *ABP1* and its neighbouring genes. Positions and directions of the various primers used in the PCR reactions are indicated. **b**, Agarose gel image of *abp1-1* genotyping results. From left to right are samples from WT, *abp1-1* heterozygous and *abp1-1* homozygous plants rescued by the *35S:At4g02990* transgene. Genotyping primers are A1, A3 and pD991-RB. Note that all of the plants contain the *35S:At4g02990* transgene. The *35S:At4g02990* transgene rescued the previously reported embryo-lethal phenotype of *abp1-1*. **c**, The *At4g02990* gene in *abp1-1* seemed to be deleted. Various PCR reactions failed to amplify the promoter region of *ABP1/At4g02990* or the coding region of *At4g02990* of *abp1-1*$^{-/-}$ with the *35S:At4g02990* transgene. For each primer pair, two DNA samples were used as templates. The left lane used DNA from *abp1-1*$^{+/-}$ with the *35S:At4g02990* transgene. The right lane referred to the DNA template from *abp1-1*$^{-/-}$ with the *35S:At4g02990* transgene. Note that the primer pairs used in this study cannot amplify the *35S:At4g02990* transgene. **d**, A schematic representation of the T-DNA insertion in *abp1-1*. The exact size of the T-DNA fragment is not known. The left border is also not defined. Based on the PCR results shown in **c**, the *At4g02990* coding region and its promoter have been deleted.
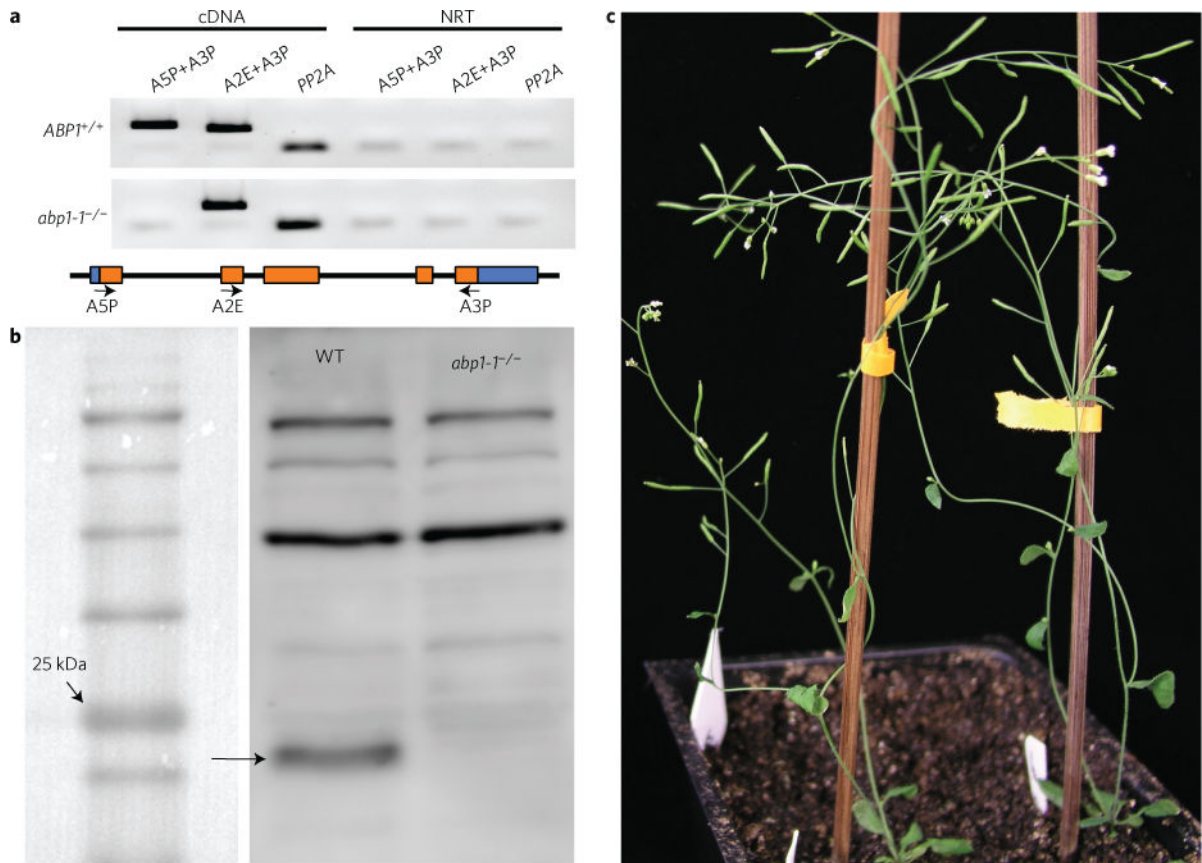
Figure 2. Characterization of *abp1-1* rescued by the *35S:At4g02990* transgene
a, RT–PCR results of WT and *abp1-1⁻/⁻*. Note that *abp1-1⁻/⁻* still produces partial *ABP1* mRNA. The relative positions of the primers are indicated. NRT: no reverse transcriptase added. *PP2A* was used as a control. **b**, Western blot results show that *abp1-1⁻/⁻* does not produce ABP1 protein (arrow). The left lane is pre-stained protein size markers that were run with the samples in the same gel. The marker lane and the sample lanes were photographed at different settings. **c**, *abp1-1⁻/⁻* plants are viable and fully fertile. Left, WT with the *35S: At4g02990* transgene. Right, *abp1-1⁻/⁻* with the *35S:At4g02990* transgene.