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Spotlight

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Auxin Binding Protein 1: A Red Herring After All?

The natural auxin indole-3-acetic acid is the first hormone identified in plants, and since it plays such a central role in plant growth and development, auxin has been the subject of intensive studies. A central question has been how the auxin signal is perceived by plant cells. The earliest experiments showed the presence of auxin binding particles at the plasma membrane (PM) and in the endoplasmic reticulum (ER) (Hertel et al., 1972). Screens for PM-localized auxin binding activities have led to the photo-affinity labeling and purification of Auxin Binding Protein 1 (ABP1) from maize coleoptile cells (Löbler and Klämbt, 1985). Despite observations in different laboratories that ABP1 localized to the PM where it seemed to mediate rapid electrophysiological and cell physiological responses to auxin, the auxin community remained skeptical about the role of ABP1 as auxin receptor for a long time, in part because of its predominant localization in the ER (reviewed by Napier et al., 2002). At some point, ABP1 was even jokingly referred to as a potential red herring in the search for the auxin receptor (Venis, 1995). However, after the first *Arabidopsis abp1-1* loss-of-function allele pointed to a key role for ABP1 in cell elongation and division, the auxin community has adopted this abundantly expressed 22-kDa protein as extracellular auxin receptor (reviewed by Napier et al., 2002). Especially in recent years, the role of ABP1 in development has become more firmly established, in part as modulator of clathrin-mediated endocytosis and microtubule orientation through its action on the Rho of Plants (ROP) family of GTPases (Robert et al., 2010; Chen et al., 2012, 2014) but also as regulator of auxin-responsive gene expression (Tromas et al., 2013). Recent evidence that auxin-bound ABP1 docks on the extracellular domain of the TRANSMEMBRANE KINASE1 (TMK1) finally linked its apoplastic localization to signaling by the PM-associated ROPs. TMK1 belongs to a small subfamily of four leucine-rich-repeat receptor-like kinases and the quadruple *tmk1234* loss-of-function mutant shows several auxin-related phenotypes (Dai et al., 2013; Xu et al., 2014). In addition, auxin-mediated activation of ROP2 and ROP6 and the downstream effects on the actin and microtubule cytoskeleton, respectively, are largely abolished in this mutant (Xu et al., 2014; Grones and Friml, 2015).

ARABIDOPSIS ABP1: A CENTRAL PLAYER IN DEVELOPMENT OR NOT?

The strong defects observed for the *Arabidopsis abp1-1null* allele, which were seemingly confirmed by the later identified *abp1-1s* allele (Table 1), have considerably hampered ABP1 research. In the homozygous state, *abp1-1* causes arrest of cell division, thereby blocking embryogenesis at the globular stage (Chen et al., 2001). In the heterozygous state, various weaker auxin-related defects have been reported, such as altered gravitropic and phototropic responses, changes in hypocotyl length, and changes in expression of early auxin-induced genes (Effendi et al., 2011). The strong phenotype of the *abp1-1* allele has triggered the isolation of a weaker allele (*abp1-5*) with a point mutation in the auxin binding pocket, and the generation of knockdown lines by the inducible expression of either antisense *ABP1* RNA or antibodies directed against ABP1 (Table 1). In a recent publication, ABP1 mutant versions with amino acid substitutions in the auxin binding pocket were expressed in the *abp1-1* background (Effendi et al., 2015). A central aspect of all these mutant lines is that they show a weak reduction in auxin sensitivity similar to heterozygous *abp1-1* mutant plants (Effendi et al., 2011). Interestingly, over expression of an ABP1 deletion version lacking the KDEL ER-retention signal also led to auxin-related phenotypes but frequently also to more severe phenotypes such as seedling lethality or sterile development (Robert et al., 2010).

In an attempt to study the role of ABP1 in flower development, Gao et al. (2015) designed an elegant CRISPR-CAS-based strategy to obtain mutant lines that become homozygous for an *abp1* null mutation at the onset of flower development. For this purpose, the *ABP1* gene-specific guide RNA was expressed under the constitutive *35S* promoter and the CAS9 endonuclease was expressed under the *APETALA 1* promoter. To their surprise, the authors did not obtain T1 plants with mutant phenotypes, and when they recovered a T2 plant homozygous for a 5 base pair (bp) deletion in the first exon (named *abp1-c1*), this plant also showed a wild-type appearance. Sequencing of RT-PCR-derived *ABP1* cDNA from this plant line confirmed that the 5 bp deletion is present in mRNA transcripts and causes a frame shift generating a premature stop codon. Western blot analysis using anti-ABP1 antibodies showed that the ABP1 protein is not detectably expressed and that *abp1-c1* is likely a *null* allele. To confirm their results, the authors obtained a T-DNA insertion line from the *Arabidopsis* stock center. RT–PCR and Western blot analysis indicated that this mutant allele (*abp1-TD*) is also a *null* mutant with the same wild-type appearance as the *abp1-c1* allele. This led the authors to conclude that ABP1 is not required in plant development, at least not under the growth conditions tested.

HOW SHOULD THE AUXIN COMMUNITY DEAL WITH THESE CONFLICTING DATA SETS?

The article by Gao et al. (2015) presents the auxin community with a dilemma. Do we trust the data accumulated by many different laboratories during 40 years of ABP1 research or do we accept the rather convincing evidence presented by Gao et al. (2015)

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Allele	Type	Description	Phenotypes	Reference
abp1-c1	5 bp deletion	CRISPR/CAS generated 5 bp deletion 107 bp downstream from ATG	Wild-type	(Gao et al., 2015)
abp1-TD1	T-DNA insert	T-DNA insert 27 bp downstream from ATG	Wild-type	(Gao et al., 2015)
abp1-1	T-DNA insert	T-DNA insert 51 bp downstream from ATG	Embryo lethal	(Chen et al., 2001)
abp1-s1	T-DNA insert	T-DNA insert in the 5′ UTR of *BSM/RUG2*	Embryo lethal	(Tzafrir et al., 2004)
abp1-5	Point mutation	TILLING selected point mutant: substitution in the auxin binding pocket	Pavement cell (PC) defects, auxin insensitive	(Xu et al., 2010)
SS12S	Knockdown	Inducible antibody against tobacco ABP1	Cotyledon defects, growth delay/arrest, sterility	(Braun et al., 2008; Tromas et al., 2009)
SS12K	Knockdown	Inducible antibody against tobacco ABP1	Cotyledon defects, growth delay/arrest, sterility, reduced auxin sensitivity	(Braun et al., 2008; Tromas et al., 2009, 2013)
ABP1AS	Knockdown	Inducible *ABP1* antisense RNA	Cotyledon defects, growth delay/arrest, PC defects, auxin insensitivity	(Braun et al., 2008; Tromas et al., 2009; Xu et al., 2010)
ABP1$^{\Delta KDEL}$-GFP	Overexpression	Overexpression of ABP1-GFP fusion lacking the KDEL domain	Reduced auxin sensitivity, seedling lethality, sterility	(Robert et al., 2010)
XVE >> ABP1 OE	Overexpression	Estradiol-inducible overexpression of ABP1-GFP	Enhanced auxin-induced microtubule re-orientation	(Chen et al., 2014)
abp1-8	Overexpression	*abp1-1* overexpressing tagged ABP1 with substitution in auxin binding pocket	Reduced auxin sensitivity, PC defects	(Effendi et al., 2015)
abp1-9	Overexpression	*abp1-1* overexpressing tagged ABP1 with substitution in auxin binding pocket	Reduced auxin sensitivity, PC defects, reduced auxin transport	(Effendi et al., 2015)
abp1-10	Overexpression	*abp1-1* overexpressing tagged ABP1 with substitution in auxin binding pocket	Reduced auxin sensitivity, PC defects, reduced auxin transport	(Effendi et al., 2015)
abp1-11	Overexpression	*abp1-1* overexpressing tagged ABP1	Near wild-type phenotypes, reduced auxin transport	(Effendi et al., 2015)

Table 1. *abp1* Loss-of-Function Alleles and *ABP1* Overexpression or Inducible Knockdown Lines.

that *ABP1* is not important for plant development? There are several aspects that should be considered before drawing a final conclusion.

First, the analysis performed by Gao et al. (2015) makes it very likely that the new mutants represent *null* alleles but it does not fully exclude that the mutant alleles produce a low level of functional ABP1, undetectable on Western blot, but sufficient to obtain a wild-type phenotype. The 5 bp deletion in the *abp1-c1* allele is close to the first intron and a small part of the mutant transcripts could be rescued by alternative splicing, which has been shown to occur for the *ABP1* gene (Wang and Brendel, 2006), e.g. by using a possible cryptic splice acceptor site a few base pairs upstream of the mutation (AGGA). It would therefore be interesting to know if more T2 lines with larger deletions in the *ABP1* gene were rescued from the CRISPR-CAS approach. Moreover, the *abp1-TD* allele has an activation tag T-DNA, containing four tandem 35S promoters on the right border (Robinson et al., 2009), inserted close to the translation start of the *ABP1* gene. While RT–PCR and Western blot analysis exclude that ABP1 is detectably produced in this line, it is still possible that a truncated transcript is produced that leads to low-level expression of a functional ABP1 protein. For both new alleles, the mutation is located in the region coding for the signal peptide, which does not require strong conservation (Martoglio and Dobberstein, 1998; Napier et al., 2002). Mutant ABP1 versions with a few amino acid deletions or substitutions in their signal peptide are therefore likely to be functional. We have to note here that this is an extremely unlikely scenario. However, if this scenario is true, this would still imply that the phenotypes observed for the *ABP1AS* antisense line (Braun et al., 2008; Tromas et al., 2009; Xu et al., 2010) are not caused by the reduced, but still detectable, *ABP1* expression.

Second, it would be good to analyze the different *abp1* mutant alleles (including *abp1-5* and *abp1-1* and *abp1-1S*) by genome sequencing to know the exact nature of the mutations and to

exclude the occurrence of gene duplications or second site mutations.

In the most likely situation that the *abp1-c1* and *abp1-TD* alleles are true *null* mutants, the strong phenotypes of the *abp1-1* and *abp1-1s* alleles could be explained by a second site mutation in another gene. In fact, the T-DNA insertion in the embryo lethal *abp1-1s* allele is located in the 5′ untranslated region of the inversely oriented *BELAYA SMERT/RUGOSA2 (BSM/RUG2)* gene located upstream of *ABP1* (Babiychuk et al., 2011; Quesada et al., 2011). Interestingly, the *bsm* mutant allele shows embryo arrest at the late globular stage (Babiychuk et al., 2011) and the fact that the *BSM/RUG2* promoter region partly overlaps with the *ABP1* coding region suggests that the embryo lethality observed for *abp1-1* and *abp1-1s* might be caused by disruption of the *BSM/RUG2* promoter function, which for the *abp1-TD* allele might be overcome by the presence of the *35S* enhancer sequences on the activation tag T-DNA. In any case, it will be essential to reevaluate the *abp1-1* complementation experiments presented in previous publications (Chen et al., 2001; Effendi et al., 2015). For the phenotypes observed in the *ABP1* antisense or antibody lines Gao et al. (2015) suggested that they could be caused by off target knockdown of other genes. It is important to note here that these off target genes could still encode redundantly acting, yet unidentified auxin receptors that may compensate for the loss of ABP1 in the *abp1-c* and *abp1-TD* alleles.

PERSPECTIVE

The publication by Gao et al. (2015) provides food for thought. Can plant life proceed without a PM-localized auxin receptor? If not ABP1, are there other (ABP1-related) auxin binding proteins at the PM that (by interacting with the TMKs) mediate the previously observed rapid cellular responses to auxin, such as elevated cytosolic calcium levels, changes in pH, or ROP-dependent changes in cytoskeleton localization or orientation (Napier et al., 2002; Shishova and Lindberg, 2010; Monshausen et al., 2011; Chen et al., 2014; Xu et al., 2014)? It is still too early to rewrite the text books, as one can be sure that several laboratories are currently investigating whether ABP1 has been a red herring after all or not. It has been suggested to ''re-examine previous data, down to the lab bench level'' (Liu, 2015). In our opinion, the most important issue is to unequivocally determine which of the reported *abp1* alleles are true *nulls* and whether there are undetected off-site mutations or unexpected effects of the known mutations that explain the observed differences between the earlier ''reference'' alleles and the new *abp1* alleles that show wild-type phenotypes.

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