Auxin-binding protein1 (ABP1), the second auxin receptor

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Abstract

Despite of knowing the 3-dimensional structure ABP1 is not fully acknowledged as an auxin receptor.We used the homozygous lethal ABP1 insertional mutant (Chen et al. 2001) which is viable in the heterozygous *abp1/ABP1* state. *abp1/ABP1* seedlings are defect in phototropism and gravitropism of roots and shoots. Those populations are composed of a major slow reacting and a minor normal reacting group. *abp1/ABP1* seedlings show strong root slanting, longer hypocotyls, and only slightly increased lateral root numbers. Root auxin responses (lateral roots,main root length) in *abp1/ABP1* seedlings are only slightly less sensitive than in wt. In short and long days *abp1/ABP1* plants flower earlier. They have more branches and decreased main stem diameter, indicating decreased apical dominance. Auxin-induced genes (qPCR of: *IAA2, IAA11, IAA12, IAA13, IAA14, IAA19, IAA20, SAUR9, SAUR15, SAUR23, GH3.5, ABP1*) respond to auxin (0.1µM/1µM/10µM) 2-10-fold stronger in wt than in *abp1/ABP1* seedlings (30 & 60 min). Auxin content and uptake of auxin in *abp1/ABP1* seedlings is not distinguishable from wt. Basipetal auxin transport in *abp1/ABP1* roots is slower than in wt. Thus ABP1 is a receptor with probable functions in auxin transport and gene regulation. The necessary functional link to TIR1-linked gene regulation could be provided by phospholipase(s) A (FEBS Lett. 2007, 581:4205-4211).

Defects in auxin-related phenotypes in heterozygous *abp1/ABP1* plants

Fig. 1

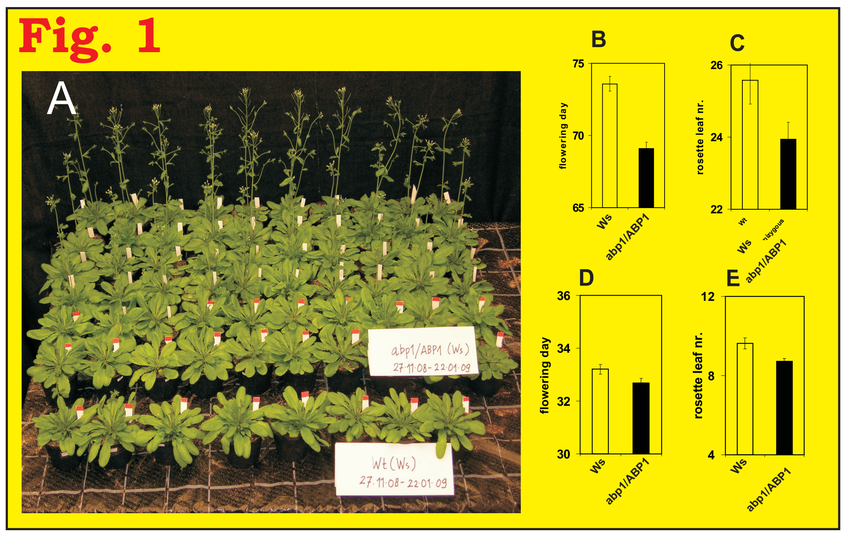


Fig. 1. Early flowering phenotype of wild type Ws and heterozygous abp1/ABP1 plants grown in short days (8/16 light/day) (A, B, C) or long days (16/8 light/day) (D E). (A) In short days grown progeny of seeds from a kanamycin-resistant heterozygous abp1/ABP1 plant (in background) and Ws wild type plants (front row). Plants were grown in short days and ordered, those with open flowers to the back and with labeled small white tags, and those not having flowers, with small red tags. (B) On short day and (D) long day, abp1/ABP1 produced flower earlier [($p<0.01$) and ($p<0.05$) respectively]. WT has more rosette leaves ($p<0.05$) on Long day (C) and also short day ($p<0.05$)(D). Flowering was recorded as first open flower. Rosette leaves and cauline leaves were counted at the time of bolting. Cauline leaf numbers were not different in all cases. All plants were PCR-genotyped.

Auxin sensitivity of *abp1/ABP1* seedling

Fig. 2

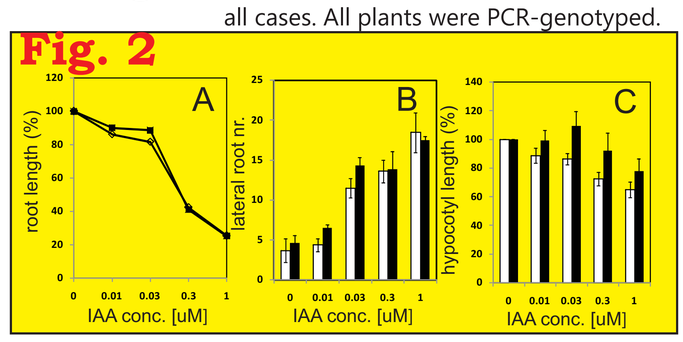


Fig. 2. Auxin sensitivity of abp1/ABP1 seedlings. All seedlings were pre-grown on vertical agar plates without auxin for 4 d, and then transferred to plates containing increasing concentrations of IAA. The abp1/ABP1 seedlings were selected as having a strong slanting angle from the segregating population after 3 d and both Ws and mutant seedlings were transferred to a fresh plate for 4 d. Response to auxin of (A) relative main root length, (B) lateral root number (C) relative hypocotyl length. (n=20 wild type Ws; n= 20 abp1/ABP1; S.E.).

Defect in apical dominant of heterozygous *abp1/ABP1* plants

Fig. 3

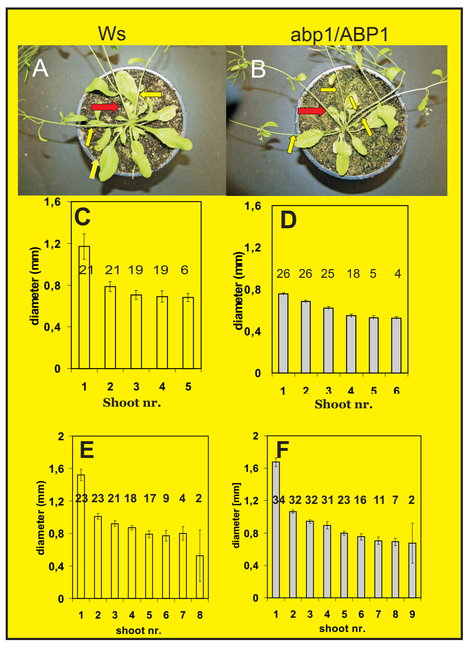


Fig. 3. Apical dominance of wild type Ws and heterozygous abp1/ABP1 plants grown in long days (16/8h light/dark) or short days (8h/16h light/dark). (A) Long day wild type Ws plant. (B) Long day heterozygous abp1/ABP1 plant. Red arrow: main stem; yellow arrows: lateral stems. Plant genotypes were determined by PCR. Note the lower number of rosette leaves and absence of prominent main stem in mutant plants.One out of three experiments is shown. (C) Stem thickness and stem number of wild type Ws long day plants (n as indicated; S.E.). (D) Stem thickness and total number of stems of heterozygous abp1/ABP1 plants (n as indicated; S.E.). (E) Stem thickness and stem number of wild type short day Ws plants (n as indicated; S.E.). (F) Stem thickness and total number of stems of short day heterozygous abp1/ABP1 plants (n asindicated; S.E.). Plant genotypes were determined by PCR. Numbers on top of bars: total number of branches for this branch class. One out of two experiments is shown.

Phenotypic appearance and responses to gravity and light

Fig. 4

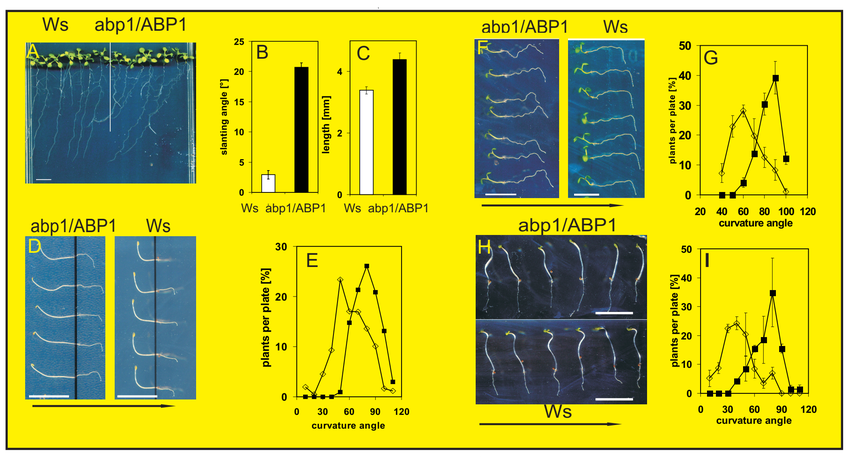
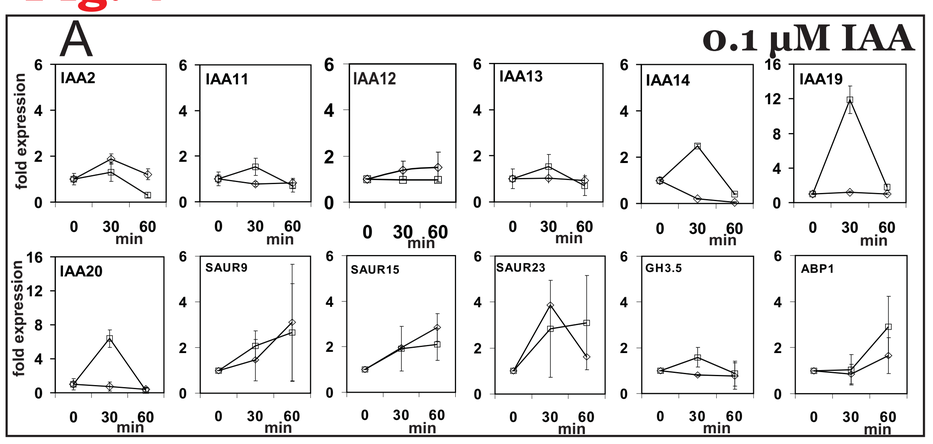


Fig. 4. Phenotypic appearance and responses to gravity and light in heterozygous abp1/ABP1 seedlings (A).Phenotype of 2 weeks old WT and heterozygous abp1/ABP1seedlings. (B) Heterozygous abp1/ABP1 produce greater slanting angles (n=20; S.E., $p<0.01$) and longer hypocotyl (n=20; S.E., $P<0.01$) (C). (D) Representative images of 4 d old seedlings after 24 h gravitropic response and (E) their graphic quantification of hypocotyl curvature angle (n=180; S.E.). (F) Representative images of 7 d old seedlings after 24 h gravitropic response and (G) their graphic quantification of root curvature angle (n=96; S.E.). (H) Representative images of 4 d old seedlings after 10 h phototropic response and (I) their graphic quantification (n=80; S.E.). Open diamonds: seeds from a kanamycin-resistant abp1/ABP1 plant; Filled squares: Ws

Regulation of early auxin-induced genes in heterozygous *abp1/ABP1* showed slightly defect in comparison to WT

Fig. 7



No different in auxin uptake between heterozygous *abp1/ABP1* and Wild type, but auxin moved slower in heterozygous *abp1/ABP1* plants in basipetal direction

Fig. 5

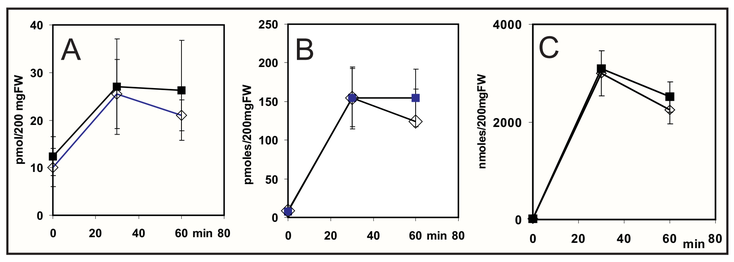


Fig. 5. Auxin uptake into wild type and abp1/ABP1 seedlings. Plants were grown, selected, and treated with auxin as described in the legend for Fig. 7. Analysis of IAA content was performed with quadrupole GC-MS system (Agilent) and results from three experiments were pooled. (A) Treatment with 0.1 µM IAA. (B) Treatment with 1 µM IAA. (C) Treatment with 10 µM IAA. Wild type plants: filled squares,abp1/ABP1:open diamonds (n= 4-6; S.D.).

Fig. 6

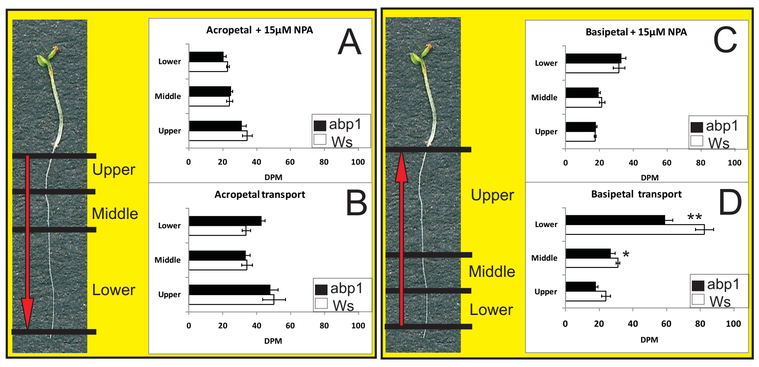


Fig.6. Polar auxin transport in wild type and abp1/ABP1 roots. (A, B) Acropetal transport in the presence of 15 µM NPA (A) or without (B). Two consecutive 5 mm pieces near the source and the residual third root piece were counted as indicated on the left side of the figure. No statistically significant differences in any segment. (C, D) No significant different in basipetal transport in the presence of 15 µM NPA (C) but there were statistically significant differences ($p<0.05$ one asterix (*) in middle segment and at <0.001 two asterixes (**) in lower segment) in basipetal transport without NPA (D)

Fig. 7 Regulation of early auxin-regulated genes and of ABP1 in light-grown wild type (Ws) and abp1/ABP1 seedlings. !4 days old seedlings were grown in ½ MS agar and treated with 0.1 µM, 1 µM, or 10 µM IAA for 30 min and 60 min. Each data were collected from 2 (B) or 3 (A, C) biological replications and 3 technical replications independently. Twelve genes (IAA2, IAA11, IAA12, IAA13, IAA14, IAA19, IAA20, SAUR9, SAUR15, SAUR23, GH3.5, ABP1) were investigated using quantitative Real Time PCR method (qRT-PCR) and the results showed that those genes are expressed 2-10-fold stronger in wt than in abp1/ABP. (A) Treatment with 0.1 µM IAA. (B) 1 µM IAA. (C) 10 µM IAA. Wild type: open squares. abp1/ABP1: open diamonds.

Conclusion: ABP1 is a powerful receptor which regulates genes, likely with a functional link to TIR1. The heterozygous abp1/ABP1 mutant is defect in responses requiring polar auxin transport.

References:

Scherer, G.F.E., Zahn, M., Callis, J. and Jones, A.M. (2007) A role for phospholipase A in auxin-regulated gene expression. FEBS Lett. 581, 4205-4211

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