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ABA catabolism generates PA, a molecule able to activate a subset of ABA receptors

ABA catabolic pathways are conventionally thought of as routes for ABA inactivation and thus reduction of the well known ABA responses to cope with stress. However, this point of view was challenged by the discovery of ABA-GE as a storage form of ABA that rapidly increases active pools of ABA under osmotic stress (Lee et al., 2006) and by the finding that some forms of hydroxylated ABA generated by ABA catabolism still maintain biological activity (Zou et al., 1995; Zhou et al., 2004). Hydroxylation at C-8' position is thought to be the predominant ABA catabolic pathway and this reaction is catalyzed by the CYP707A family cytochrome P450 monooxygenases (P450s) (Krochko et al., 1998; Marion and Poll 2005) (Figure 1). 8'-hydroxy ABA is isomerized spontaneously to phaseic acid (PA), involving internal cyclization of the 8'-hydroxyl moiety onto the enone at C-2' position. This ring closure occurs in concert with the initial hydroxylation of ABA in both the in vitro and in vivo assays (Krochko et al., 1998); however this reaction is reversible and a differential biological activity between 8'-hydroxy ABA and PA has been reported (Jadhav et al., 2008). Finally, the subsequent conversion of PA to dihydrophaseic acid (DPA) is catalyzed by a soluble PA reductase (PAR) that reduces the 4'-ketone to 4'-hydroxyl group.

This PAR had remained elusive until the recent breakthrough of Weng et al., (2016). Chemical reactions similar to PAR are catalyzed by dihydroflavonol 4-reductase (DFR)-like NAD(P)H-dependent reductases and accordingly, Dr. Noel's group screened for potential candidates through a phylogenetic analysis of the DFR-like reductase family. Only one candidate was found in seed plants, which was named ABH2 for ABA hypersensitive 2 upon phenotype analysis of *abh2* T-DNA mutant lines. Catabolism of ABA is predicted to be impaired in *abh2*, which might explain the enhanced sensitivity to ABA during early seedling development. However, because PA levels are predicted to increase in *abh2* mutants, Weng et al reasoned that PA directly inhibits germination in an ABA-like manner. Additionally, the authors examined slow and fast response to drought stress in *abh2-1* plants. They found that rapid control of

leaf transpiration was not affected in *abh2-1*; however after a 2-week drought stress period *abh2-1* plants displayed improved drought tolerance.

The levels of ABA and its catabolites, i.e. PA, DPA and its 4'-O-β-glucoside (DPAG), were measured in 2-d-old wt and *abh2-1* seedlings by HPLC-MS. First at all, they found that *abh2-1* contains very little DPA compared to wt but much higher levels of PA, which is consistent with the hypothesis ABH2 being the PAR. Indeed, recombinant ABH2 reduces PA to DPA using NADPH as cofactor. Second, ABA levels in wt and *abh2-1* were comparable which discounts the hypothesis that elevated PA levels in *abh2-1* might result in feedback inhibition of the CYP707A family P450s. Therefore, the enhanced sensitivity of *abh2-1* to ABA during early seedling development might reflect an ABA-like effect of PA or PA-specific effects that are inhibitory for germination. Previous researchers have studied the capability of PA to inhibit germination, induce stomatal closure and ABA-responsive genes, but conclusive results have not been obtained yet to support a clear ABA-like effect of PA in different plant species. Thus, Walker-Simmons et al., (1997) concluded that PA lacked inhibitory effect on wheat germination at concentrations 50-fold higher than ABA but induced an ABA-responsive LEA gene. Hill et al., (1992) found that PA was about 10% as effective as ABA as a germination inhibitor of barley immature embryo. Sharkey and Raschke (1980) found that PA can cause stomatal closure in some species but it is barely effective in barley and maize. Moreover, rates of CO_2 assimilation were not affected by PA or DPA but were reduced markedly by ABA. Weng et al., performed a transcriptomic profiling of Arabidopsis treated with 50 μM PA and found that most PA-responsive genes overlapped with ABA-responsive genes; however, differentially expressed genes in response to PA were about 10% of those in response to ABA and they were less altered upon PA treatment than upon ABA treatment. Therefore, 50 μM PA possesses ABA-like activity on gene expression but PA's effect is markedly lower than the dramatic change induced by ABA treatment on plant transcriptome.

To further increase PA levels in Arabidopsis, Weng et al., crossed the *abh2-1* mutant with plants that overexpressed the biosynthetic PA enzyme CYP707A3. Thus, blocking PA degradation together with enhancing biosynthesis leads to high PA levels and reduced ABA content. These plants do not show retarded growth as observed in some ABA biosynthetic mutants, which suggests a compensatory effect of PA under conditions of low ABA. ABA metabolite profiling over a 20-d drought period revealed that PA levels increased circa 10-fold at day 12 and then remained relatively constant until day 20, whereas ABA levels also increased markedly (at least 2-fold over PA

levels) at day 12 and then declined at the end of the drought period. Thus, PA might be able to extend or prolong some ABA effects; however, simultaneous ABA action is also required to cope with drought stress because high PA levels combined with reduced ABA content did not up-regulate the ABA-responsive genes *RD29B* and *HIS1-3* neither improve water content in leaves after drought treatment (Weng et al., 2016).

Weng et al., determined whether the PYR/PYL/RCAR family of ABA receptors recognized PA as an effective ligand using the PP2C inhibition assay. ABA was more effective than PA to inhibit PP2C activity for all the eleven recombinant receptors that were tested. This result is in agreement with previous results from Kepka et al., (2011), who found a moderate effect of PA in regulating ABI2 activity in the presence of RCAR1/PYL9, RCAR3/PYL8 and RCAR11/PYR1. However, Kepka et al., did not find a significant effect of 3 μM PA in inhibiting germination, inducing stomatal closure or inhibiting root growth of Arabidopsis La-*er*. Weng et al., found that some ABA receptors, PYL8, PYL9 and PYL11, are barely sensitive to PA. Other receptors, PYR1, PYL1 and PYL10, are circa 40-fold less sensitive to PA than ABA, and in the case of PYL2, PYL4, PYL5 and PYL6, they were between 10-20 fold less sensitive. Finally, PYL3 has an IC50 value only 4-fold higher for PA than ABA. PYL3 has a very specific expression in the chalazal seed coat and might sense PA during regulation of seed development or germination; however PYL3 is barely expressed in the rest of plant tissues (http://jsp.weigelworld.org/expviz/expviz.jsp). To further pinpoint potential receptors able to recognize PA in vivo, the *abh2-1* p35S:CYP707A3 plants were crossed with individual *pyr/pyl* mutants. The authors found that *pyl2-1* (La-*er*)/*abh2-1 p35S:CYP707A3* plants showed stunted growth and decreased drought stress torelance, which suggests that PYL2 is an important receptor for PA. Intriguingly *pyl5-1 abh2-1 p35S:CYP707A3* does not seem to show this phenotype, even though PYL5 was 8-fold more effective than PYL2 (IC50 0.56 μM and 4.5 μM, respectively) in inhibiting PP2C activity in response to PA.

A crystal structure was obtained of PYL2 in complex with PA and PYL3-PA-HAB1 ternary complex. PA induces a conformational change in PYL2 that might promote disassociation of the dimeric receptor as well as closure of one gating loop around the ligand. The bulkier head group of PA pivots circa 60º relative to the PYL2-ABA corresponding structure; however both PA and ABA adopt similar conformations when comparing PYL3-PA-HAB1 and PYL3-ABA-HAB1 complexes.

In summary, the results from Weng et al., provide a key advance towards our understanding of ABA catabolism and the ability of PA to activate a subset of ABA

receptors. Further evaluation of the biological effects PA and 8'-hydroxy ABA in different plant species will be required to obtain a comprehensive picture on the role of these ABA catabolites as hormonal regulators.

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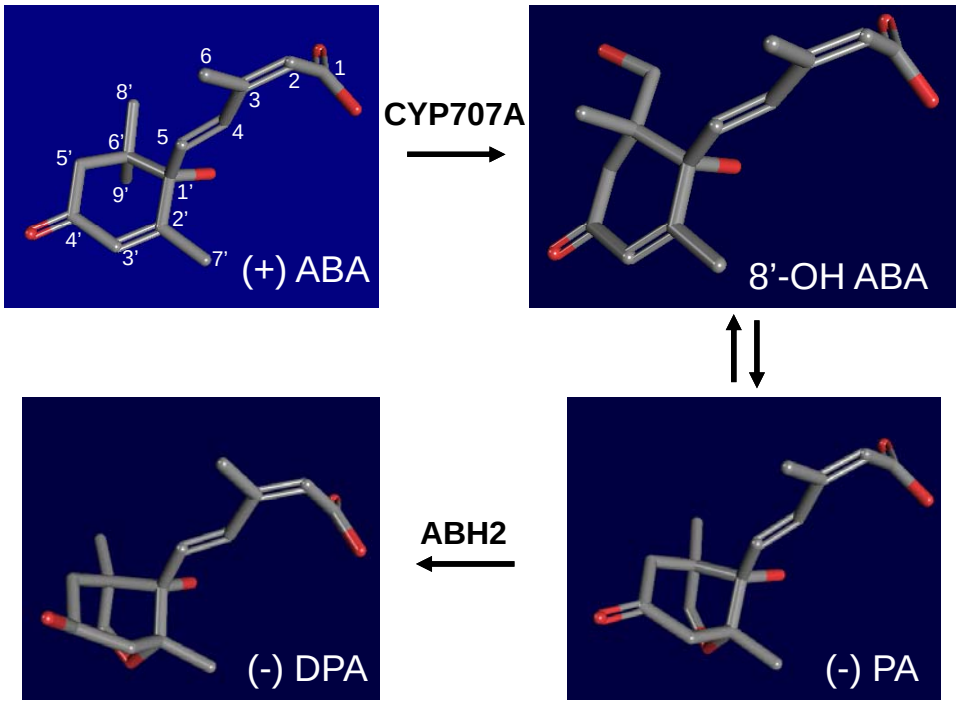

Figure 1. Catabolism of ABA through the 8'-hydroxylation pathway. ABA, abscisic acid; 8'-OH ABA, 8'-hydroxy ABA; PA, phaseic acid; DPA, dihydrophaseic acid; CYP707A, CYP707A family cytochrome P450 monooxygenases; ABH2, PA reductase. Cyclization of 8'-OH ABA into PA is a reversible reaction.