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New players in ABA signaling: identification of PUB12/13 involved in degradation of ABA co-receptor ABI1

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Abscisic acid (ABA) was originally identified in the early 1960s, and was thought to be involved in fruit and leaf abscission. Over the past 50 years, ABA was intensely studied and has become one of the vital plant hormones implicated in seed dormancy, seed germination, stomatal regulation, flowering, senescence and environmental stress responses. Early work isolated the mutants *abi1* (*ABA-insensitive 1*) and *abi2*, which are insensitive to ABA [1]. These two genes were subsequently revealed to encode members of the clade A protein phosphatase 2Cs (PP2Cs) of serine/threonine protein phosphatases (see review [2]). Additional members of the clade A PP2Cs such as A*BA-hypersensitive germination1 (*AHG1) and AHG3, were also shown to participate in ABA stress signaling [2]. These PP2Cs function as negative regulators of ABA response.

In searching for ABA receptors, Park et al. and Ma et al. [3,4] demonstrated that *PYRABACTIN RESISTANCE* 1 (*PYR1*) and regulatory components of ABA receptor (RCAR) proteins bind ABA to mediate ABA-dependent inactivation of two PP2Cs, ABI1 or ABI2. The PYR/PYL/RCAR proteins belong to the START protein family, which is necessary for both pyrabactin and ABA signaling [4]. The ABA-PYLs binding affinities increase when PYLs interact with PP2Cs; thus, PP2Cs are considered as ABA co-receptors [5]. The crystallography-based structural study of PYL2-ABA-PP2C complex uncovers a conserved gate-latch-lock mechanism for ABA signaling [5]. The interaction of ABA, PYL2 and PP2C relieves PP2C inhibition of protein kinases SnRK2.2/2.3/2.6 (SnRK2.6 is also named OST1), GHR1, SnRK1 and some calcium-dependent kinases. These kinases phosphorylate and activate the downstream targets such as ABRE BINDING FACOTR (ABF) transcriptional factor and the anion channel SLAC1, which regulate a broad range of developmental processes (reviewed in [2]).

Although ABA perception and signaling pathway have been well elucidated, whether there exist other factors that modulate this pathway remains to be explored. A recent study identified two U-Box E3 ligases, PUB12 and PUB13, which interact with ubiquitinate ABI1, a key PP2C in ABA signaling cascade [6]. Based on the 26S proteasome inhibitor assay, Kong et al. firstly show that the turnover of ABI1 is mediated by proteasome pathway. To identify the ABI1-interacting E3 ubiquitin ligase, the authors performed yeast two-hybrid screening and eventually focused on two plant U-box E3 ligases, PUB12 and PUB13. Co-immunoprecipitation (Co-IP), LC-MS/MS analysis of ABI1 interacting partners and a luciferase complementation imaging assay confirmed that ABI1 interacts with the ARM domain of PUB12/13.

Next, an *in vitro* ubiquitination assay was employed to test the roles of ABA, PUB12/13 and PYR/PYLs in ABI1 ubiquitination. Their results show that PUB12/13 is required for ubiquitinating ABI1. In the presence of PYR1, ABA is necessary for ABI1 turnover; in contrast, in the presence of PYL4/9, ABA is not required for ABI1 ubiquitination.

To further test the roles of PUB12/13 and ABA in ABI1 degradation *in vivo*, the authors examined the ABI1 accu-

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mulation in the wild-type and *pub12pub13* mutant plants treated with and without ABA. ABI1 degradation is compromised in the *pub12pub13* mutant as compared with the wild-type, suggesting that PUB12/13 is required for ABI1 turnover. The authors also co-transfected the *p35S::PYR1-Flag Arabidopsis* protoplasts with *p35S::ABI1-Myc* and *p35S::PUB13-Flag* constructs, and then treated the cultured protoplast cells with and without ABA. Their result shows that ABA treatment promotes ABI1 degradation.

Because ABI1-PYLs interaction is essential for PUB12/13-mediated ubiquitination of ABI1, the authors hypothesized that ABI1 degradation would be reduced in the *pyl* mutant. Immunoblot analysis of ABI1 protein levels in the wild-type and *pyl1pyl2pyl3pyl4* mutant reveals suppressed ABI1 degradation in the *pyl*quadruple mutant compared with that in the wild-type, suggesting that ABA-bound PYLs are required for ABI1 degradation.

To investigate whether the *pub12pub13* mutant has compromised ABA response, the authors conducted a few assays, which consistently show that PUB12/13 is involved in ABA signaling pathway. First, OST1 kinase activity is reduced in the *abi1-1*(Col) and *pub12pub13* mutants compared with that in the wild-type. Second, *pub12pub13* mutant exhibits enhanced ABA-insensitive phenotypes such as cotyledon greening and inhibition of root growth compared with the wild-type. Third, RNA-seq analysis of the wild-type, *abi1-1*(Col) and *pub12pub13* mutants reveals downregulation of many ABA-induced marker genes in the *pub12pub13* mutant compared with those in the wild-type. The expressions of ABA-induced marker genes in the *abi1-1* (Col) and *pub12pub13* mutants have strong correlation, which implies that the impaired ABA sensing in the *pub12pub13* mutant is likely caused by accumulation of ABI1. Fourth, the *pub12pub13* mutant produces less H_2O_2 in the guard cells, loses more water and has impaired stomatal movement relative to the wild-type.

The above results indicate that PUB12/13 binds ABI1 and mediates ABI1 ubiquitination. Genetically, ABI1 should act downstream of PUB12/13. To test this notion, the authors introduced the *abi1-3* loss-of-function allele into the *pub12pub13* mutant and investigated ABA response of the *abi1-3pub12pub13* triple mutant. Several assays consistently show that the triple mutant recovers the ABA insensitive phenotype of the *pub12pub13* mutant.

In summary, Kong et al. reported the identification of U-box E3 ligases PUB12 and PUB13 that interact with ABI1, which inhibits downstream protein kinases such as OST1 (upper panel, Figure 1). ABA and its receptor PYR1 bind ABI1 to relieve ABI1 inhibition of OST1 (middle panel, Figure 1). PUB12/13 mediates ABI1 ubiquitination by two routes: (i) ABA-dependent pathway requires ABA-PYR1-ABI1 interaction (middle panel, Figure 1); (ii) ABA-independent pathway involves PYL9-ABI1 interaction (bottom panel, Figure 1). This model illustrates the machinery of ABA induced signaling pathway. However, a few questions remain to be resolved, *e.g.*, how PUB12/13 initiates ABI1 ubiquitination after interacting with PYR1/PYL9? Could ABA-PYR1-ABI1 interaction induce ABI1 confirmation change that leads to ABI1 degradation? Could ABA-PYR1 and PYL9 compete to bind ABI1? Is there any other component involved in ABA-PYR-ABI1 induced signaling? PUB12/13 is known to target FLS2 in immune signaling, then, is there any cross-talk between ABA signaling and innate immune response pathways? Answering these questions will get deeper insights into the ABA triggered signaling mechanism in plants.

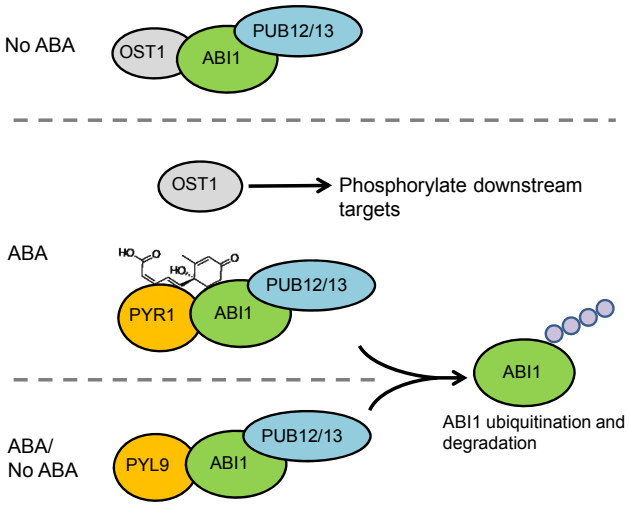

Figure 1 A proposed model for ABA signaling and PUB12/13 mediated ABI1 ubiquitination pathways.

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