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# Novel Pathway Regulates ABA Perception: How ESCRTs Regulate Stability of ABA Receptors

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## Commentary

The phytohormone Abscisic Acid (ABA) participates in a vast variety of physiological processes of plants, such as stress defenses, embryo development, vegetative growth and flowering [1-4]. With decades of studies, our knowledge of ABA signaling has tremendously increased, especially with the identification of Pyrabactin Resistance/Pyrabactin Resistance-Like/Regulatory Components of the ABA Receptor (PYR/PYL/RCAR) type ABA receptors [5-7]. The well-recognized ABA signaling cascade has been clearly illustrated, which consists of PYR/PYL/RCAR type ABA receptors, type 2C Protein Phosphatases (PP2Cs), SNF1-Related protein Kinases2s (SnRK2s) and a series of substrates of SnRK2s [8,9]. The turnover of ABA receptors by 26S proteasome are well documented but the new discoveries about the endomembrane trafficking pathway in ABA signaling regulation are not well summarized. We believe the deep understanding about the complexity of receptor turnover could benefit the whole signaling research field.

Recent studies have described different types of E3 ligases that regulate ABA receptors stability in nucleus and plasma membrane. In nucleus, both the substrate adapter of Cullin4-RING E3 ubiquitin Ligases (CRL4s), Deetiolated1 (DET1)- and Damaged DNA Binding protein1 (DDB1)-Associated1 (DDA1), and the F-box protein of the Skp1-Cullin1-F-box (SCF) complex, the RCAR3 Interacting F-box Protein 1 (RIFP1), can recognize the ABA receptor PYL8 (also named RCAR3), and then accelerate PYL8 degradation via the 26S proteasome [10,11]. Different from the multiunit E3 ubiquitin ligases CRL4s and SCF complex in nucleus, the single unit RING-type E3 ligase Ring finger of Seed Longevity1 (RSL1) mediates ubiquitination of the ABA receptors PYR1/PYL4 on the plasma membrane [12]. Thus, the 26S proteasome-dependent degradation pathway indeed subtly modulates the turnover of ABA receptors in both cytosol and nucleus. Interestingly, the interaction of RSL1 with PYR1/PYL4 at Trans-Golgi Network (TGN)/Early Endosome (EE) indicated that the endomembrane trafficking pathway might also regulate the turnover of ABA receptors [12].

Although the non-26S-proteasome endomembrane trafficking pathway is evolutionarily conserved and important from yeast to mammals [13], the understanding of this pathway is extremely limited in plants, especially in the aspect of the plant hormone signaling. In plants, only very few of membrane receptors have been found to be modified by K63 polyubiquitination, which serves as a signal for modified proteins sequestered into vacuoles/lysosomes and degraded by luminal proteases, instead of the 26S proteasome [14].

In the vacuoles/lysosomes mediated degradation pathway, the Endosomal Sorting Complex Required for Transports (ESCRTs) function essential roles in recognizing and sorting the ubiquitinated cargo proteins, and biogenesis of Multivesicular Bodies (MVBs) that are responsible for taking the cargo proteins into the vacuoles/lysosomes for degradation in the late stage of the trafficking pathway [13,15]. ESCRTs consist of ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III complexes in both yeast and mammals [13], while ESCRT-0 has not been found in plants as yet [13]. Vacuolar Protein Sorting 23A (VPS23A) that belongs to the ESCRT-I possesses a Ubiquitin-conjugating Enzyme Variant (UEV) domain, missing a catalytic cysteine in the Ubiquitin-Conjugating (UBC) enzyme domain, but still retains the ubiquitin-binding ability [16].

In our recent publication, we have found Arabidopsis ESCRT-I unit VPS23A negatively regulates the ABA signaling pathway [17]. The vps23a null mutant showed an extremely hypersensitive phenotype in comparison to Wide-Type (WT) in terms of both cotyledon greening and root growth under ABA treatment. Cell biological assay showed that both VPS23A and PYR1/PYL4 co-localized with endosome sorting markers, including the TGN marker MANI, the EE marker SCAMP1 and the Late Endosome (LE) marker ARA7. As expected, VPS23A and PYR1/PYL4 co-localized in the endosomal compartments. Biochemical assay found that VPS23A could directly interact with both the ABA receptors and the K63-linked ubiquitin. It is known that K63-type polyubiquitination is an important signal for cargo proteins recognized by ESCRTs in the endosomal trafficking pathway [18]. It was supposed that K63 ubiquitinated PYL4 on the plasma membrane might be internalized, and then directed by VPS23A toward lysosome for degradation or storage.

We further found that VPS23A could accelerate the degradation of PYL4, and the degradation rate of PYL4 significantly slowed down with E64d (an inhibitor of lysosomal/vacuolar hydrolases) treatment. The PYL4 protein increased in the vps23a null mutant compared to WT, meanwhile, the PYL4 degradation rate decreased. Thus, the ESCRT-I unit VPS23A actually destabilizes ABA receptors via endosome-mediated vacuolar degradation pathway (Figure 1). The phenotype of the vps23a combined with pyr/pyl quadruple mutants turned out to be ABA-insensitive, as same as the quadruple mutants, this genetic evidence further supports above conclusion, and thus demonstrates that VPS23A takes a key role of endocytosis in ABA signaling at the perception step.

Coincidentally, almost at the same time Rodriguez's lab also reported the Arabidopsis atypical ESCRT-I unit FYVE-domain protein Required for Endosomal sorting1 (FREE1, also named FYVE1) could interact with PYL4 and enhanced its degradation via vacuolar

degradation pathway [19]. FREE1 is a phosphatidylinositol-3-phosphate-binding protein, and it can be recruited to LE [20,21]. FREE1 interacted with PYL4, actually the PYL4-RSL1 complex was required for FREE1 mediated PYL4 degradation. Additionally, FREE1, VPS23A and an ESCRT-III unit SNF7A are found to be co-localized in endosomal membranes. Those data suggested that different ESCRTs units might cooperate in the PYL4 degradation processes [19].

Taken together, given the lacking of ESCRT-0 in higher plants and the two units of ESCRT-I VPS23A and FREE1 both interact with ABA receptors and mediate their degradation through endomembrane trafficking pathway, there could be two possibilities for the cooperation between VPS23A and FREE1. VPS23A and FREE1 probably directly recognize the PYR/PYL/RCAR type ABA receptors independently [17,19]; or VPS23A may accept the ABA receptor cargoes after FREE1 recruiting them [19].

Overall, plants precisely adjust the turnover of ABA receptors and regulate the ABA signaling by both the 26S proteasome and the vacuolar degradation pathways. This kind of modulation may contribute to the plant flexible regulation of ABA signaling for adapting to different environmental conditions [17,19,22]. The finding that ESCRTs participate in the turnover of ABA receptors through endosome-mediated vacuolar degradation pathway brings us a breakthrough in the understanding of the regulatory mechanisms of ABA signaling. However, it still exists a few questions that should be addressed in future, such as what the key signal is in determining the specificity of different degradation pathways, and whether RSL1 is the E3 ligase for the ubiquitination of the target recognized by VPS23A in vacuolar degradation pathway.

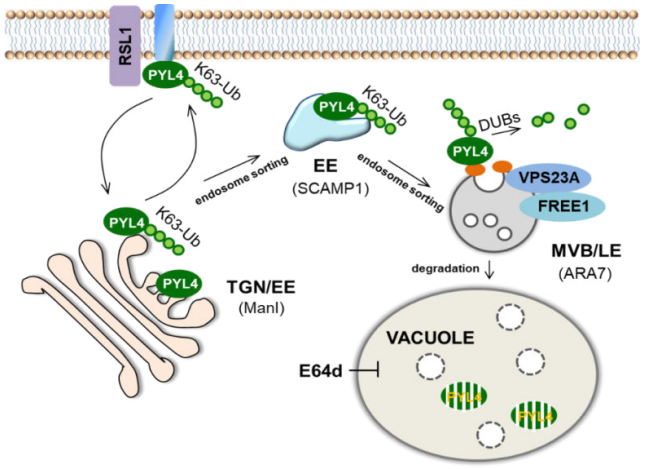


**Figure 1:** ESCRT-I Units VPS23A and FREE1 Regulate PYL4 Degradation via Endosomal Trafficking Pathway.

The ABA receptor PYL4 is ubiquitinated by E3 ligases in the plasma membrane and Trans-Golgi Network (TGN)/Early Endosome (EE) such as RSL1. Subsequently, the ESCRT-I units VPS23A and FREE1 recognize the K63 ubiquitinated form of PYL4 and recruit it into the Multivesicular Body (MVB) toward the TGN, the EE and late endosome (LE) step by step via ESCRTs-mediated vacuolar trafficking pathway. Before being transported into the vacuole, the K63 ubiquitinated PYL4 would be deubiquitinated by deubiquitinating enzymes (DUBs). Eventually, PYL4 arrives at the vacuole for degradation. This process can be blocked by the protease inhibitor E64d.

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