ABSTRACT

Abscisic acid (ABA) signaling plays a critical role in regulating root growth and root system architecture. ABA-mediated growth promotion and root tropic response under water stress are key responses for plant survival under limiting water conditions. In this work, we have explored the role of *Arabidopsis* (*Arabidopsis thaliana*) PYR/PYL/RCAR receptors (PYRABACTIN RESISTANCE1 (PYR1)/PYR1 LIKE (PYL)/REGULATORY COMPONENTS OF ABA RECEPTORS) for root ABA signaling. As a result, we discovered that PYL8 plays a nonredundant role for the regulation of root ABA sensitivity. Unexpectedly, given the multigenic nature and partial functional redundancy observed in the PYR/PYL family, the single *pyl8* mutant showed reduced sensitivity to ABA-mediated root growth inhibition. This effect was due to the lack of PYL8-mediated inhibition of several clade A phosphatases type 2C (PP2Cs), since PYL8 interacted in vivo with at least five PP2Cs, namely HYPERSENSITIVE TO ABA1 (HAB1), HAB2, ABAINSENSITIVE1 (ABI1), ABI2, and PP2CA/ABA-HYPERSENSITIVE GERMINATION3 as revealed by tandem affinity purification and mass spectrometry proteomic approaches.

Membrane-delimited abscisic acid (ABA) signal transduction plays a critical role in early ABA signaling, but the molecular mechanisms linking core signaling components to the plasma membrane are unclear. We show that transient calciumdependent interactions of PYR/PYL/RCAR ABA receptors with membranes are mediated through a 10-member family of C2-domain ABA-related (CAR) proteins in Arabidopsis thaliana. Specifically, we found that PYL4 interacted in an ABA-independent manner with CAR1 in both the plasma membrane and nucleus of plant cells. CAR1 belongs to a plant-specific gene family encoding CAR1 to CAR10 proteins, and bimolecular fluorescence complementation and coimmunoprecipitation assays showed that PYL4-CAR1 as well as other PYR/PYL-CAR pairs interacted in plant cells. The crystal structure of CAR4 was solved, which revealed that, in addition to a classical calcium-dependent lipid binding C2 domain, a specific CAR signature is likely responsible for the interaction with PYR/PYL/RCAR receptors and their recruitment to phospholipid vesicles. This interaction is relevant for PYR/PYL/RCAR function and ABA signaling, since different *car* triple mutants affected in *CAR1, CAR4, CAR5, and CAR9* genes showed reduced sensitivity to ABA in seedling establishment and root growth assays. In summary, we identified PYR/PYL/RCAR-interacting partners that mediate a transient Ca^{2+}-dependent interaction with phospholipid vesicles, which affects PYR/PYL/RCAR subcellular localization and positively regulates ABA signaling.