Keeping the Ethylene Response Fluid: GDSL Lipase MHZ11 Modulates Sterol Levels and Ethylene Signaling in Rice Roots

Nature’s simplest alkene, ethylene, is a gaseous phytohormone produced in planta at certain developmental stages and in response to abiotic and biotic stress. Three decades of elegant genetic, molecular, and biochemical studies conducted in the dicotyledonous model plant Arabidopsis thaliana have shed much light on ethylene biosynthetic, signaling, and response pathways (Bakshi et al., 2015). Much less is known about ethylene signaling in monocotyledonous plants (Yang et al., 2015). Here, a recent study by Zhao et al. (2020) provides novel mechanistic insight into the regulation of ethylene signaling in rice, a monocot cereal crop species.

Exogenous ethylene reduces root growth, a phenotype commonly leveraged in genetic screens to identify components of ethylene response. Zhao and colleagues trolled for mutants displaying abnormal root growth in the presence of ethylene and fish† mao huzi11 (mhz11; “mao huzi” meaning “cat whiskers” in Chinese). The authors used map-based cloning to target mhz11 to a candidate gene that encodes a putative GDSL lipase. An additional, independent allele and functional complementation confirmed that this GDSL lipase gene corresponded to mhz11. A detailed series of transgenic and biochemical experiments were used to localize the MHZ11 protein to the ER membrane, where the ethylene receptors and a subset of signaling proteins also reside (Bakshi et al., 2015). The authors showed that ethylene induced MHZ11 expression in shoots and roots through the canonical ethylene signaling pathway. Furthermore, overexpression of MHZ11 resulted in shorter roots in air or ethylene, which could be suppressed by treatment with 1-MCP, an ethylene perception inhibitor.

How might this ER-localized MHZ11 lipase facilitate ethylene signal transduction in rice roots? To address this question, Zhao and colleagues used lipidomics to identify a number of phospholipids, including phosphatidylcholine, as possible MHZ11 substrates. A phospholipase A2 enzyme assay demonstrated that MHZ11 could hydrolyze phosphatidylcholine, whereas the MHZ11(S39A) mutant protein, with a disrupted active site, decreased its hydrolase activity. The observation that plants expressing MHZ11(S39A) could not recover the mhz11 ethylene-insensitive phenotype indicated that the acyl-hydrolyzing activity of MHZ11 is necessary for its role in ethylene responses, providing key insight into the role of MHZ11 in ethylene signal transduction.

The ethylene signaling pathway as currently defined is essentially linear—where does MHZ11 fit in? Zhao and colleagues used extensive genetic interaction analyses to place MHZ11 function provisionally at the same level as the ethylene receptors, upstream of the OsCTR2 serine/threonine protein kinase, which is a conserved negative regulator of the ethylene pathway (Yang et al., 2015). Through an impressive series of experiments that included: 1) generating a specific polyclonal antibody to OsCTR2 and determining OsCTR2's subsequent phosphorylation; and 2) producing a catalytically inactive OsCTR2 and demonstrating its inability to suppress ethylene responsive genes activity, the authors were equipped to decipher the effect of MHZ11 on OsCTR2 phosphorylation status in vivo in response to ethylene. Time-course monitoring of OsCTR2 phosphorylation in wild-type, mhz11 mutant, and MHZ11-overexpression (MHZ11-OE) roots in response to ethylene or 1-MCP indicated an essential role of MHZ11 in promoting ethylene inhibition of OsCTR2 phosphorylation/activity. Returning to genetics, the authors found that null or dominant gain-of-function alleles of MHZ11 or OsERS2, respectively, led to constitutive phosphorylation of OsCTR2. These observations suggested that both MHZ11 and OsERS2 are required for ethylene inhibition of OsCTR2 phosphorylation. Epistasis analysis utilizing MHZ11-OE and a dominant OsERS2 gain-of-function allele indicated that MHZ11 functions at the level of the ethylene receptor.

A remaining fundamental question Zhao et al. (2020) sought to answer was what specific lipid species is involved in disrupting OsERS2-OsCTR2 interaction and OsCTR2’s subsequent phosphorylation? The authors investigated free sterol, a primary bilayer component maintained by sterol acylation and known to regulate membrane fluidity (Lindsey et al., 2003). Free sterol levels in roots were elevated in mhz11 mutant and decreased in MHZ11-OE plants. The mhz11 mutant doubly treated with ethylene and FEN, a sterol biosynthesis inhibitor, blocked OsCTR2 phosphorylation, partially restoring ethylene response in roots. Importantly, FEN disrupted OsERS2-OsCTR2 interactions in vivo as revealed by bimolecular fluorescence complementation and split luciferase complementation assays.

The authors presented a model summarizing their results. In response to ethylene, MHZ11 lipase activity maintains low sterol levels on the ER membrane and potentially disrupts OsERS2-OsCTR2 interaction, thereby inhibiting OsCTR2 phosphorylation, and hence activity, thus activating the ethylene response.
This exciting work provides crucial mechanistic insight into the regulation of ethylene signaling in a monocot species and opens the door for future studies that carry potential to improve agriculture.

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Plant Cell; originally published online March 19, 2020; DOI 10.1105/tpc.20.00218

This information is current as of May 6, 2020