On the Inside

Nitro-Fatty Acids in Plant Signaling

Nitric oxide (NO), the major reactive nitrogen species in plants, is involved in a wide range of plant functions, including seed germination, development, senescence, and responses to biotic or abiotic stresses. Nitro-fatty acids (NO_2-FAs) are produced when reactive nitrogen species derived from NO react with unsaturated fatty acids. In animal systems, NO_2-FAs are potent signaling mediators involved in anti-inflammatory responses. In this issue, **Mata-Pérez et al. (pp. 686–701)** examine the endogenous occurrence of nitro-linolenic acid (NO_2-Ln) in Arabidopsis (*Arabidopsis thaliana*) and the modulation of NO_2-Ln levels throughout development. A transcriptomic analysis by RNA-seq technology established a clear signaling role for this molecule, and demonstrated that NO_2-Ln was involved in plant defense responses against different abiotic stress conditions, mainly by inducing heat shock proteins as they do in animal systems. NO_2-Ln levels rose significantly under several abiotic stress conditions such as wounding or exposure to salinity, cadmium, and low temperature. The observed picomolar levels of NO_2-Ln in Arabidopsis are consistent with its proposed role as a signaling effector.

UV Avoidance Behavior of the Nucleus

Some plant cells cope with strong light by relocating their organelles. For example, in leaves of Arabidopsis, the nuclei of mesophyll and pavement cells are positioned at the center of the cell bottom in the dark and relocate to the side walls within 1 h of continuous irradiation with strong blue light. Analysis of Arabidopsis mutants indicated that the side-wall nuclear positioning is regulated by the blue-light receptor phototropin2 and by the actin cytoskeleton. The mechanism of the side-wall positioning of nuclei is different, however, from the mechanism of dark-induced positioning. The cell-bottom positioning of nuclei is regulated by actin as well as the plant-specific motor myosin XI-i. In myosin *XI-i* mutants, the cell-bottom positioning is aberrant but the side-wall positioning occurs normally. A question that remains, however, is what are the functional roles of these nuclear movements? In this issue, **Iwabuchi et al. (pp. 678–685)** provide evidence that nuclear movement is a rapid and effective strategy to avoid ultraviolet B (UVB)-induced damages. When mesophyll cell nuclei were positioned on the cell bottom in the dark, the sudden exposure of these cells to UVB caused severe DNA damage and cell death. The damage was remarkably reduced in both blue-light-treated leaves and mutant leaves defective in the actin cytoskeleton. Intriguingly, in plants grown under high-light conditions, the mesophyll nuclei remained on the side walls even in the dark. Thus, Arabidopsis has two strategies for reducing UVB exposure: rapid nuclear movement against acute exposure and nuclear anchoring to the side walls in response to chronic exposure.

Smells Emitted by the Sensitive Plant: A Touchy Subject

The roots of the sensitive plant *Mimosa pudica* emit a pungent, unpleasant sulfurous odor when dislodged from the soil. This mechanosensitive response is selective: touching the roots with soil or human skin resulted in odor release, but agitating the roots with other materials such as glass failed to induce a similar response. Chemical analyses by **Musah et al. (pp. 1075–1089)** reveal that the stench is associated with the enhanced emission of a subset of organosulfur compounds when the roots are stimulated. Light and scanning electron microscope imaging studies revealed the presence of sac-like protuberances along *M. pudica* seedling roots that collapsed when the roots were exposed to stimuli that elicited odor emission. Elemental analysis of these projections by energy-dispersive x-ray spectroscopy revealed that they contained higher levels of K^+ and Cl^- compared with the surrounding tissue. Exposing these protuberances to stimuli that caused odor emission resulted in reductions in the levels of K^+ and Cl^- in the touched area. Unresolved is the mechanism whereby these root protuberances respond to touch by humans but not by a glass rod.

Glutaredoxins Control Primary Root Growth

Most plants are wholly dependent on soil reserves of inorganic or organic nitrogen to support their growth. Thus, most current agricultural practices call for substantial inputs of chemical fertilizers that are rich in inorganic nitrogen, typically in the form of nitrate (NO_3^-), ammonium (NH_4^+), or both. To better understand how nitrate and ammonium differentially affect plant metabolism and development, **Patterson et al. (pp. 989–999)** performed transcriptional profiling of the shoots of ammonium-supplied and nitrate-supplied Arabidopsis plants. Seven genes encoding class III glutaredoxins were found to be strongly and specifically induced by nitrate. Several of the nitrate-induced glutaredoxins act as negative regulators of primary root growth. Nitrate induction of glutaredoxin gene expression was dependent upon cytokinin signaling. Moreover, cytokinins activated glutaredoxin gene expression independent of plant nitrate status. The authors propose that the glutaredoxin/cytokinin pathway may work in concert with other well-characterized programs to modulate root system architecture to maximize plant utilization of nitrate-rich regions of the soil.

Hypoxia-Induced Calmodulin-Like Protein

The exposure of plants to oxygen deprivation stress as a result of flooding, waterlogging, or poor soil aeration leads to depression of respiration, reduced adenylate energy charge, accumulation of toxic metabolites, and cytosolic acidification. A number of metabolic cues and signaling pathways are involved in triggering the hypoxia response, including cytosolic calcium signals. Ca^{2+} is a ubiquitous secondary messenger and is involved in responses to myriad developmental, hormonal, and environmental cues in plant cells. Initial evidence for a role of Ca^{2+} in hypoxia responses came from pharmacological studies that revealed that pretreatment with Ruthenium Red (RR), an inhibitor of

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organellar Ca^{2+} release, followed by hypoxia inhibited increases in *ALCOHOL DEHYDROGENASE1* transcript and impaired poststress survival of the seedlings. These studies support a role for calcium signaling as a component of the hypoxia response. Ca^{2+} signals are decoded by a collection of Ca^{2+} sensor proteins that have Ca^{2+}-binding domains that detect micromolar changes in cytosolic Ca^{2+} and mediate downstream responses. The most well-characterized of these is the ubiquitous Ca^{2+} sensor calmodulin. Higher plants, however, have a large collection of calmodulin-like proteins (CMLs) that have diverged from calmodulin in structure and function. Among the core hypoxia response proteins in Arabidopsis is the Ca^{2+} sensor CML38. *CML38* transcripts are up-regulated more than 300-fold in roots within 6 h of hypoxia treatment. Transfer DNA insertional mutants of *CML38* show an enhanced sensitivity to hypoxia stress, with lowered survival and more severe inhibition of root and shoot growth. By using yellow fluorescent protein translational fusions, **Lokdarshi et al. (pp. 1046–1059)** have found that CML38 protein is localized to cytosolic granule structures similar in morphology to hypoxia-induced stress granules, which act as mRNA storage and triage centers during hypoxia. RR treatment results in the loss of CML38 signal in cytosolic granules, suggesting that Ca^{2+} is necessary for stress granule association. These results confirm that CML38 is a core hypoxia response Ca^{2+} sensor protein and suggest that it serves as a potential Ca^{2+}-signaling target within stress granules that accumulate during flooding stress responses.

PHABULOSA Mediates Auxin Signaling

Root vascular patterning depends on class III homeodomain Leu zipper (HD-ZIP III) transcription factors (TFs) composed of five genes, including *PHABULOSA* (*PHB*). In root cells, their activity domains are regulated through the activity of microRNA165 and microRNA166. These microRNAs move from their site of synthesis in the endodermis and restrict HD-ZIP III transcripts to the central stele. This results in higher levels of the HD-ZIP III proteins in the central stele compared with its periphery. Analyses of various combinations of double, triple, and quadruple mutants as well as microRNA165-insensitive gain-of-function mutants suggest that HD-ZIP III TFs act redundantly. These HDZIP III TFs govern the patterning of the xylem axis in a dose-dependent manner: high levels promote metaxylem cell identity in the central axis, and low levels promote protoxylem at its flanks. It is unclear, however, by what mechanisms the HD-ZIP III TFs control xylem axis patterning. In this issue, **Müller et al. (pp. 956–970)** present data suggesting that HD-ZIP III TFs affect the expression of genes encoding core auxin response molecules. One of the HD-ZIP III TFs, PHB, directly binds the promoter of *MONOPTEROS* (*MP*)/*AUXIN RESPONSE FACTOR5*, a key factor in vascular formation, as well as *IAA20*, encoding an auxin/indole acetic acid protein that is stable in the presence of auxin and able to interact with and repress MP activity. The double mutant of *IAA20* and its closest homolog *IAA30* forms ectopic protoxylem, while overexpression of *IAA30* causes discontinuous protoxylem and occasional ectopic metaxylem, similar to a weak loss-of-function *mp* mutant. The authors suggest that PHB, possibly together with other HD-ZIP III TFs, focus the auxin response within the xylem axis by activating *MP*, along with its repressors, *IAA20* and *IAA30*, thereby stabilizing the vascular patterning process.

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