

PLANT SIGNALING

Unveiling the molecular details of plant signaling

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This Editorial Guide describes emerging areas of signaling research for plants. Advances in this area are key to preserving nature and maintaining the planet's health while feeding a growing human population.

A major challenge facing the world is feeding the world's growing population with the finite resources on a planet undergoing environmental change. Signaling research is essential to achieving this goal. The challenges faced by researchers of plant signaling are somewhat different than those faced by researchers interested in translating reductionist knowledge into an understanding of human physiology. Although the mustard plant *Arabidopsis thaliana* is a key model organism for unraveling plant physiology and how plants cope with abiotic and biotic stresses, analysis in agriculturally relevant species is also necessary. Compared with researchers studying animals or even microbes, plant researchers have fewer cell culture systems and so face the greater challenge of performing detailed analysis of cellular processes in the intact plant. This has been both a benefit and a challenge. Many regulatory molecules are defined by characterization of a genotype-to-phenotype output, so their physiological importance is generally undisputed. But discerning the molecular regulatory connections is more difficult in the context of the whole plant, especially when the growing parts of plants have highly dynamic cellular populations that rely on spatial and temporal context for function. For example, the shoot and root meristems cannot be studied *ex planta*. Instead, as exemplified by the study of Li *et al.* in their analysis of the regulation of auxin signaling by the transcription factor LEAFY in flower formation, *in vivo* analysis is the key. Elsayad *et al.* reported the application of fluorescence emission–Brillouin imaging, a sophisticated imaging technique, to investigate the regulation of the subcellular mechanical properties of live plants.

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Like animals, plants must also tolerate highly variable environmental conditions, which include both abiotic stress (such as dehydration, depletion of nutrients, and changes in temperature and light) and biotic stress (such as herbivory, pathogen infection, and competition with parasitic plant species). Furthermore, plants cannot move to find nutrients or reproductive partners or escape predation or stress. Thus, many plants rely on symbiotic relationships with other organisms to provide key nutrients for growth and produce signals to encourage beneficial interactions with other species or deter harmful ones. Plants lack mobile cells that move throughout the plant through the circulatory system. Instead, plants rely extensively on the regulated biosynthesis, transport, and metabolism of peptides and metabolites, and each cell must be able to respond to pathogenic infection. The complexity of plant hormonal systems is still being discovered, with many receptors and peptides still “orphans” and new paradigms in the mechanisms of hormone sensing and signal transduction being revealed. The complexity in hormonal signaling is high, with regulation occurring at the level of hormone transport (for example, in auxin signaling), and at the level of biosynthesis (for example, when part of the biosynthesis of a hormone) occurs in one part of the plant and then the intermediate is transported to the target tissue, where biosynthesis is completed and the receptors for the hormone are present.

Science Signaling publishes research defining the differences between plants and other species, the mechanisms by which plants interact with pathogenic and nonpathogenic species, the pathways that control plant growth and development, and the molecular events by which plants adapt to environmental conditions. This Editorial Guide highlights some of the plant research published in the journal in the past few years, which includes basic research about biochemical mechanisms

of regulation, systems-level analysis of regulatory processes, and technical advances for investigating plant signaling.

In this issue, Urano *et al.* investigate the evolution and function of plant Gα proteins. Whereas, through gene duplication followed by gradual evolution, animals evolved multiple classes of closely related Gα subunits that mediate distinct cellular responses, plants have a single class of canonical Gα proteins that is homologous to animal Gα_s, but also have a family of extra-large Gα (XLG) proteins. Their analysis indicates that the diversification of the XLG proteins coincided with the colonization of land by plants. Phenotypic analyses of *Arabidopsis* Gα and XLG mutants revealed that canonical Gα signaling is primarily involved in developmental processes, whereas XLG proteins preferentially mediate stress responses. Thus, the rapid diversification of the XLG proteins may have helped plants withstand the new stresses encountered in the terrestrial environment. The Review by Jeandroz *et al.* explores the evolution of nitric oxide (NO) signaling in plants. In animals, NO is mainly produced by NO synthases, a family of enzymes partly conserved in bacteria. In contrast, of the 1300 plant species analyzed, only a few algae had proteins with similarity to NO synthases, and land plants apparently lack this mechanism of producing NO. Thus, NO signaling has evolved differently in plants than animals, indicating that insights gleaned from the animal kingdom about this process may not inform plant research.

NO signaling involves the posttranslational modification of target proteins, commonly, at least in animals, through S-nitrosylation of cysteine residues. Castillo *et al.* detected tyrosine nitration of receptors (called PYR/PYL/RCAR) for the hormone abscisic acid (ABA) in plants by mass spectrometry and found that this posttranslational modification, rather than S-nitrosylation, inhibited their ABA-induced activity *in vitro*. ABA inhibits plant growth and promotes adaptive stress responses. Tyrosine nitrosylation of PYR/PYL/RCAR proteins is an irreversible covalent modification, which resulted in the polyubiquitylation and proteasomal degradation of the receptors. The authors proposed that tyrosine nitration, which requires both NO and superoxide anions, is a rapid mechanism by which NO limits ABA signaling under conditions in which NO and reactive oxygen species are both produced.

ABA binding to PYR/PYL/RCAR inhibits phosphatases of the clade A type 2C protein

phosphatase family (PP2Cs) and increases the phosphorylation of ion channels and transcription factors by protein kinases of the SnRK2 family. L eran *et al.* identified ABI2, a phosphatase that is inhibited by ABA in response to stress, as a key positive regulator of the nitrate (NO₃⁻) transporter NPF6.3. ABI2 dephosphorylated components of a calcium-sensing kinase complex that phosphorylated and inhibited NPF6.3-dependent NO₃⁻ uptake, sensing, and signaling in roots. Because, when dephosphorylated by ABI2, a related calcium-sensing kinase complex also stimulates K⁺ influx, the identification of this ABA-regulated phosphatase suggests a mechanism for integrating regulation of ionic balance and energy-consuming NO₃⁻ reduction during periods of plant stress.

The regulation of ion channel activity by ABA signaling is the key to plant responses to drought. One way that plants survive drought conditions is by closing gas exchange pores called stomata. Drought stimulates production of ABA, which stimulates the opening of anion channels to reduce the volume of guard cells so that they come together and the stomata close. Maierhofer *et al.* expressed various kinases and their interacting partners in *Xenopus* oocytes along with the anion channels SLAC1 or SLAH3 and determined that, in addition to the two previously known types of kinases that activated these channels, a third type of kinase also stimulated channel opening. In addition to the ion channels on the plasma membrane, the movement of water and ions into and out of the vacuole contributes to the turgor pressure that controls stomatal opening and closing. Wege *et al.* found that *Arabidopsis* plants lacking the vacuolar anion/proton exchanger AtCLCa did not effectively open or close their stomata. Light stimulated AtCLCa-mediated uptake of anions into the vacuole to aid in opening the pores, and ABA stimulated AtCLCa phosphorylation and enhanced its efflux activity, which helped to close the pores. This study revealed that a single vacuolar exchange protein can move ions bidirectionally depending on the plant's photosynthetic and water conservation needs.

Umezawa *et al.* took an omic approach to explore ABA signaling and identified direct and indirect targets of the ABA-activated kinases of the SnRK2 family. By comparing the phosphoproteomes of wild-type and triple-mutant plants lacking all SnRK2 family members to dehydration or exogenous ABA, the authors found that dehydration stress regu-

lated fewer SnRK2-dependent phosphorylation events than did ABA. These examples of physiological and biochemical processes controlled by ABA signals illustrate the complexity in studying plants.

Although plants lack an adaptive cell-based immune system, they have a robust immune response and use chemical signals to interfere with microbial pathogens and parasitic plants. Plants also use chemical signals to attract organisms needed for plant growth and reproduction or to repel harmful ones. For example, plants use volatile chemicals to attract pollinating insects and repel herbivores. Corral-Lugo *et al.* determined that a plant compound, rosmarinic acid, functioned as a plant-derived mimic of an activator of the bacterial quorum-sensing pathway, which bacteria use to regulate group behaviors, such as the formation of biofilms. Rosmarinic acid bound to and activated RhlR, a transcriptional regulator in the quorum-sensing pathway of the plant and human pathogen *Pseudomonas aeruginosa*. This study shows how plant research can be important for both agriculture and biomedicine, because identification of such a plant compound could be used to strategically disrupt signaling that enables bacteria to be pathogenic to plants and humans.

Receptors of the leucine-rich repeat receptor-like kinase (LRR-RLK) and receptor-like kinase (RLK) families regulate many plant processes, including responses to pathogens, growth signals, and self-incompatibility signals. These represent a very large and complex family of proteins that form homooligomeric and heterooligomeric complexes and exhibit complex cellular dynamics. Similar to receptor tyrosine kinases and serine-threonine kinase receptors in animals, the LRR-RLKs and RLKs signal through phosphorylation cascades. Somssich *et al.* compared the dynamics of the CLAVATA1 receptor complexes (CLV1 and CLV1-CRN), which bind the meristem-regulating peptide CLV3, and the flagellin receptor complex (FLS2-BAK1), which binds the bacteria-derived peptide flg22, using multiparametric fluorescence imaging microscopy. Their analysis revealed that in the absence of flg22, the flagellin receptor subunits were kept apart and that the addition of flg22 triggered receptor dimerization and then oligomerization of the dimeric complexes. In contrast, the receptors for CLV3 existed as complexes before the presence of the ligand, and CLV3 induced their aggregation into membrane subdomains. This difference may enable the continuous signaling necessary for

CLV3-dependent stem cell homeostasis and prevent inappropriate inhibition of plant growth and development by the flagellin receptor by requiring that receptor complexes form only in the presence of ligand.

Science Signaling not only strives to serve both the plant and animal signaling communities, but also to facilitate serendipitous discovery between these communities by highlighting signaling themes that are common and distinct between these kingdoms. The editors encourage readers to think of themselves as signalers first, and we, the editors, raise awareness of findings with interdisciplinary potential by highlighting such studies in Editors' Choice (see Gough and VanHook).

Related Resources

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