Update on Receptors and Signaling

The ability of cells to sense and respond to endogenous cues as well as cues from neighboring cells and tissues underlies the life and death of cells, their growth and proliferation, differentiation into tissues, and ultimately development into morphologically diverse organisms. Being able to sense and respond to an ever-changing environment allows organisms to thrive, propagate, and overcome life-threatening challenges and thus is critical for their survival as individuals and as species. The study of signal transduction in plants has expanded dramatically from the early efforts to define the basic components of signaling by the most classically known hormones (e.g. auxin, abscisic acid, ethylene, and brassinosteroid) and environmental stresses. These studies have led to paradigms for different molecular strategies and provided the foundation for recent efforts to study the expanding world of signals, such as endogenous peptides, and inform the need to integrate linearity in established signaling pathways into connected networks. Breakthroughs in approaches, aided by escalating computational power, have enabled plant signal transduction research to advance with unprecedented breadth and depth, addressing diverse processes to reveal, for example, the dynamic of signaling and structural perspectives at the single-molecule and atomic levels. This Focus Issue on Receptors and Signaling addresses some of the most important advances and new landmarks in the field. In addition to commissioned Updates by experts in their respective topics, several research articles highlight recent accomplishments in these areas.

Ubiquitination/26S proteasome-regulated proteolysis, firmly established for auxin signaling (Leyes 2018), continues to be identified as central to an ever-increasing number of signaling pathways. Tal et al. (2020) provide an overview of major hormone signaling pathways mediated by regulated proteolysis. This review also focuses on the recently identified phytohormone strigolactone, whose receptor DWARF14 is surprisingly also an enzyme that converts its ligand into nonbioactive products, and highlights recent structural insights. The classical topics in hormone signaling, such as for auxin and abscisic acid, continue to be areas of active research. Fernandez et al. (2020) report additional complexity in abscisic acid signaling mediated by regulated proteolysis in different subcellular compartments. Ramos Baez et al. (2020) demonstrate the use of synthetic and heterologous systems to dissect the molecular mechanisms of auxin signaling in maize (Zea mays). This latter system is transferable and potentially useful for functional analysis of putative auxin signaling components from new sources.

How the ubiquitous second messenger Ca$^{2+}$ is decoded into specific responses remains an enigma. Liu et al. (2020) report a theoretical simple gene expression system to uncover a design principle with which the Ca$^{2+}$ signal is decoded into specific gene expression responses.

Signaling in defense responses has seen the most explosive advances in the last two decades. Li et al. (2020) provide an interesting overview on how studies of plant immunity have come into their own over the decades with the identification of common grounds with animal immunity as well as unique strategies and components. The list of danger signals, including pathogen-associated molecular patterns (PAMPs) from microbes and pests (nonself) and damage-associated molecular patterns (DAMPs) from damaged host cells (modified self), is expansive and still growing. Plants employ both cell surface-localized and intracellular immune receptors to detect nonself and modified-self molecules and activate immune responses. Surface-localized immune receptors, also called pattern recognition receptors (PRRs), are either receptor kinases (RKs; which include a ligand-binding ectodomain, a single-pass transmembrane domain, and an intracellular kinase domain) or receptor proteins (RPs; which share a similar domain organization with RKs but lack an intracellular kinase domain). Pathogens can be sensed by nucleotide-binding leucine-rich repeats (NLRs) that recognize directly or indirectly secreted proteins inside host plant cells.

Recent structural studies have uncovered the molecular mechanisms underlying how plant immune receptors perceive their ligands and become activated. These studies, reviewed by Wang and Chai (2020), illustrate how different types of PRR ectodomains mediate the perception of distinct microbial or plant-derived ligands and how ligand binding induces complex formation with coreceptors that are required for the activation of downstream immune signaling. Recent studies, including those based on cryo-electron microscopy, have demonstrated that some plant NLRs form supramolecular structures, so-called resistosomes (analogous to the inflamasome built by mammalian NLRs), that are required for immune-induced cell death. The study of plant PRRs has also highlighted general principles of RK/RP-based signaling. Albert et al. (2020) review different types of plant PRRs and their ligands, how PRR complexes are regulated, and current knowledge of immune signaling downstream of plant PRRs. The area remains extraordinary. For instance, one of the best studied plant PRRs is the Leu-rich RK FLS2 that perceives the PAMP flg22, a conserved peptide epitope from bacterial flagellin. Collins et al. (2020) show that the abundances of Arabidopsis (Arabidopsis thaliana) FLS2 and

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its coreceptor BAK1/SERK3 are controlled by the trans-Golgi network-associated clathrin adaptor EPSIN1, which correlates with a positive role of EPSIN1 in antibacterial immunity against the plant pathogen Pseudomonas syringae. Li et al. (2020) discuss the growing number of identified and potential plant DAMPs, including extracellular ATP (eATP), extracellular NADP [eNAD(P)], and their receptors and/or candidate receptors. For instance, plant PRRs can detect modified-self molecules such as eATP. The first example of a plant eATP receptor is an L-type lectin RK, P2K1 (Choi et al., 2014). As reviewed by Li et al. (2020), L-type lectin RKs are also potential receptors for eNAD(P).

Important gaps still exist in our knowledge of how plant cells are able to distinguish between foes (pathogens) and friends (beneficials), especially when plant receptors and microbial ligands involved in root symbioses with nitrogen-fixing rhizobial bacteria or mycorrhizal fungi show striking similarities with those involved in PRR-mediated immune signaling. Chiu and Paszkowski (2020) provide a comprehensive review of current knowledge of the ligands, receptors, and downstream signaling involved in the perception of symbionts and the establishment of symbioses that ultimately contribute to an optimal plant nutrition.

Following its first identification in yeast, the evolutionarily conserved Target of Rapamycin (TOR) has been functionally characterized in yeast, plants, and mammals. An atypical Ser/Thr protein kinase, TOR mediates protein phosphorylation to integrate different internal and external signals to control multiple developmental processes as well as plant responses to a variety of stresses. Focusing on plant adaptation to nutrient deficiency and abiotic stresses, Fu et al. (2020) review the mechanisms of two major ways that TOR signaling adapts to abiotic stresses in plants: cross talk with abscisic acid signaling and inducing autophagy. They also consider potential regulators and downstream targets of TOR and reflect on future research directions. With a broader perspective, Lamers et al. (2020) consider how plants survive under an ever-changing environment where signals are abundant, diverse, and often simultaneous. This article reviews current knowledge for sensing different abiotic stresses, ranging from extremes in temperature and water supplies. It also evaluates the status of established and recently introduced sensors and/or potential sensors and considers a potential nexus that integrates and dispatches signals to cellular responses.

An area of rapid growth is in the identification of large and diverse families of peptides as signals. In the past decade, the biological functions of these signal peptides have been widely studied, and many are involved in the development of different cell types/organs/tissues, such as CLE peptides in vascular differentiation and stem cell fate determination, and in various cell-cell communication processes, such as Rapid Alkalinization Factor (RALF) peptides that play roles in plant-pathogen and male-female interactions. Receptors for these peptides are still emerging and, together with their peptide ligands, a number have been structurally characterized, considerably advancing our understanding of the activation and signal transduction of receptor-peptide complexes. The importance of this emerging area is underscored by several Updates and a research article. Fukuda and Hardtke (2020) review the importance of CLE peptide-related signaling pathways during development of the vascular tissues in Arabidopsis. They address the complexity of multiple intersecting pathways as well as their interaction with plant hormones and environmental stresses. Jourquin et al. (2020) highlight the diverse roles played by CLE peptides in lateral root initiation. They also discuss a multitude of other peptides, diverse in structure but all important to the final root architecture, to highlight that current knowledge about peptide involvement in development is only the tip of the iceberg. In their research article, Berckmans et al. (2020) examine the CLE40 peptide, which in differentiated columella cells is perceived by the ACR4-CLV1 receptor complex. It was previously hypothesized that blocking the movement of the transcription factor WOX5 from the quiescent center to the adjacent cell layer regulates the differentiation of the columella stem cells. Berckmans et al. (2020) report findings that lead them to propose an alternate model in which the main function of CLE40 is to correctly position the quiescent center where WOX5 functions, supporting the idea that the regulation of root stem cells is achieved by the complex interaction of two antagonistic pathways.

RALFs are a family of peptide regulators discovered in the early 1980s that reemerged in recent years as an area of intense interest. This is largely driven by their identification as ligands for the FERONIA RK and its coreceptor, the glycosylphosphatidylinositol-anchored protein LLG1. FERONIA RK is a member of the CrRLK1L RK family and has been reviewed extensively in recent years (Li et al., 2016; Franck et al., 2018) and in several Updates here. Blackburn et al. (2020) weave a historical perspective on the discovery of RALFs with an account of the current status of these peptides as ligands for CrRLK1L RK-LLG coreceptor complexes. They review different functional roles mediated by these RALF-RK complexes and current mechanistic understanding about the signaling processes from a large number of biological, biochemical, and structural studies.

Many plant RKs, diverse in sequence and physiological functions, require a different RK as a coreceptor to perceive and transmit signals, and these coreceptors are often common to different pathways. In general, coreceptors have a much smaller extracellular domain compared with their ligand-binding RK partners. Gou and Li (2020) discuss recent progress and outstanding questions in paired RK-mediated signal transduction pathways and their roles in plant development. They reviewed BAK1 and homologous SERKs and their partnership with at least nine different RKs in a variety of signaling pathways controlling myriad processes. Other partnerships, such as CLV3 signaling...
and involving coreceptors CLAVATA3 INSENSITIVE RECEPTOR KINASES (CIKS), are also discussed.

As knowledge mounts and methods improve in sophistication, the obvious consideration for researchers focused on signaling processes is to obtain an understanding that closely approximates the actual environment where these processes take place. In this regard, together with the cell wall, the plasma membrane is a critical barrier that determines the identity of a cell and its communication strategies with the outside world. Evidence suggests that, in plants, the plasma membrane with membrane-embedded receptors is highly compartmentalized and lateral segregation of proteins and lipids is critical for cell surface signaling, modulating signal perception, specificity, and integration. Lipid-protein interaction in membranes has started to attract attention from plant scientists in relation to its role in membrane protein complex assembly, stability, and function. These effects have been attributed mainly to interactions of the receptors with lipids mediated by protein scaffolds. It is believed that physiologically relevant processes occurring in membranes involve an intense coordination of multiple lipid-protein interactions. Since the organization and dynamics of membranes have considerable impact on membrane protein structure and function, the development and characterization of experimental tools to analyze these aspects of membranes assume significance in plant research. Jaillais and Ott (2020) review the current knowledge of membrane nanodomains in the plant plasma membrane and their functional importance. They also assess the roles of lipids, the cell wall, and the cytoskeleton in shaping this diverse plasma membrane landscape and discuss plausible scenarios for the functional importance of protein nanoclustering in signal transduction.

Plants encode hundreds of putative cell surface receptors and thousands of secreted peptides that can be potentially their ligands. The study of how natural ligands influence the behavior of receptors can be challenging, as there is an enormous number of possible peptide ligand-RK pairs, ligands can be promiscuous in their binding, and one receptor can recognize several peptides. Moreover, different ligand-receptor interactions often trigger a plethora of outcomes, which depend on the cellular context. Therefore, assessing ligand-receptor interactions requires more integrative and quantitative approaches, including biophysical methods to measure membrane receptor dynamics and oligomerization state upon ligand binding. Sandoval and Santiago (2020) overview the state-of-the-art in vitro ligand-binding assays used to investigate receptor-ligand interactions. Among these, new structure-based ligand-binding technologies have emerged as powerful methods to uncover key residues and conformational changes of the receptors upon ligand binding. Together with new instruments and technologies, the options are expanding for protocols that use small amounts of unmodified receptor proteins and ligands.

We hope that in capturing a snapshot of recent achievements in the field, the work presented here will inspire future efforts while also underscoring the impact of cumulative knowledge from a large research community in a rapidly advancing field. With a number of articles submitted to the Focus Issue still at various stages of the review process, we anticipate that this Focus topic will be further enriched in the near future by these and other studies in related areas recorded in the online collection.

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