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LigandReceptor-Mediated Regulation of Growth in Plants

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Contents

Abstract

as0005 Growth and development of multicellular organisms are coordinately regulated by various signaling pathways involving the communication of inter- and intracellular components. To form the appropriate body patterns, cellular growth and development are modulated by either stimulating or inhibiting these pathways. Hormones and second messengers help to mediate the initiation and/or interaction of the various signaling pathways in all complex multicellular eukaryotes. In plants, hormones include small organic molecules, as well as larger peptides and small proteins, which, as in animals, act as ligands and interact with receptor proteins to trigger rapid biochemical changes

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and induce the intracellular transcriptional and long-term physiological responses. During the past two decades, the availability of genetic and genomic resources in the model plant species, *Arabidopsis thaliana*, has greatly helped in the discovery of plant hormone receptors and the components of signal transduction pathways and mechanisms used by these immobile but highly complex organisms. Recently, it has been shown that two of the most important plant hormones, auxin and abscisic acid (ABA), act through signaling pathways that have not yet been recognized in animals. For example, auxins stimulate cell elongation by bringing negatively acting transcriptional repressor proteins to the proteosome to be degraded, thus unleashing the gene expression program required for increasing cell size. The "dormancy" inducing hormone, ABA, binds to soluble receptor proteins and inhibits a specific class of protein phosphatases (PP2C), which activates phosphorylation signaling leading to transcriptional changes needed for the desiccation of the seeds prior to entering dormancy. While these two hormone receptors have no known animal counterparts, there are also many similarities between animal and plant signaling pathways. For example, in plants, the largest single gene family in the genome is the protein kinase family (approximately 5% of the protein coding genes), although the specific function for only a few dozen of these kinases is clearly established. Recent comparative genomics studies have revealed that parasitic nematodes and pathogenic microbes produce plant peptide hormone mimics that target specific plant plasma membrane receptor-like protein kinases, thus usurping endogenous signaling pathways for their own pathogenic purposes. With biochemical, genetic, and physiological analyses of the regulation of hormone receptor signal pathways, we are thus just now beginning to understand how plants optimize the development of their body shape and cope with constantly changing environmental conditions.

s0005

1. INTRODUCTION

p0005

Unlike animals, plants live their entire life at the place where they are first anchored. Thus, they have evolved mechanisms to adapt to their environments by creating unique body patterns and/or continually adjusting their growth rate. To do so, plants utilize ligand–receptor systems that perceive environmental changes and initiate various types of immediate responses. The first chemical compound that was proposed to be a growth stimulatory hormone found in plants many decades ago was an indole derivative, auxin (indole-3-acetic acid, IAA). IAA was first isolated from human urine then subsequently identified from plant extracts (references in Enders & Strader, 2015). Auxin induces changes in body pattern development in response to sunlight to maximize capturing the light energy and optimize growth and reproduction. After the subsequent discovery of other key small molecule plant hormones such as cytokinins (an adenine

derivative), ethylene (a gas molecule), gibberellin (diterpenoid), and abscisic acid (ABA) (synthesized from carotenoid pathway), plants were traditionally, for over several decades, believed to regulate cell physiology by synthesizing and transporting only small organic hormone-like molecules. These plant-specific signaling compounds were found to initiate signal transduction by interacting with their receptor proteins localized in the cytoplasm, nucleus, or endomembranes, instead of the more commonly known plasma membrane-bound receptor kinases found at the cell surface of animal cells. It is only last 15 years or so that plant scientists realize that like animals, plants use a wide variety of peptides and small protein growth factors as extracellular signals that interact with cell-surface protein kinases, to regulate growth. The notion that plants use peptide-based signals as important regulatory pathways that alter protein phosphorylation is consistent with the fact that receptor-like kinases (RLKs) are encoded by a very large gene family in all plants. In the best-characterized model plant, *Arabidopsis thaliana*, there are over 1000 genes encoding protein kinases, out of a total of over 25,000 protein coding genes in the genome. About half of the protein kinases in plants are membrane-bound receptor-like protein kinases which contain a single transmembrane domain with an extracellularly located ectodomain where signaling molecules bind. Among those RLKs, biological functions have been defined for only a few dozen based largely on the phenotypes observed with genetic knockout mutants. Since there are only a handful of pairs of ligand–receptors with known roles in the regulation of plant growth and development, there is a rich opportunity for future research to reveal many previously unknown pathways. For this goal, our lab has been developing and applying modern genomic technologies, including mass spectrometric-based tools that quantify changes in protein phosphorylation on a proteome-wide scale.

s0010 ## 2. NOVEL AND UNIQUE PLANT SIGNALING PATHWAYS

s0015 ### 2.1 Cytokinin

p0010 In contrast to animals, plant cell division is restricted to a small group of cells at the very tip of the root and shoot, called a meristem. At the meristem, cells are maintained undifferentiated and primarily function to produce daughter cells. Once new cells are produced, they expand in size, which contributes to organ growth. This cell division is stimulated by cytokinin, a plant hormone that is a modified adenine derivative. Kinetin is a naturally occurring cytokinin that was first isolated and characterized at the University of

Wisconsin-Madison by Miller and Skoog (Miller, Skoog, Okumura, Von Saltza, & Strong, 1956). It is important to note that this plant growth substance is *completely* different from the better-known class of proteins called "cytokines" that are involved in many aspects of animal physiology including immune system, growth, and maturation.

p0015 Cytokinin acts through a family of receptors called histidine protein kinases, one of which was first isolated in plants via a genetic screen that identified cytokinin-insensitive plants, leading to the identification of the gene as encoding a histidine protein kinase, *cre1* (cytokinin response 1) (Inoue et al., 2001). Histidine protein kinases are unique in plants, fungi, and bacteria and have not yet been found to play a role in animals. Cytokinin signals are thought to be perceived in the sensor domain of the receptor and transmitted to a kinase domain, in which the conserved histidine residue is phosphorylated. The phosphate group is then transferred to the aspartate residue of response regulator proteins, as first described in the osmosensor *EnvZ* of bacteria (Mahonen et al., 2006; West & Stock, 2001). Affinity measurements of a naturally occurring cytokinin, zeatin, as well as a synthetic analog, N^6-benzyladenine, to the receptors indicate that AHK3 appears to have the highest affinity (1.3 nM equilibrium dissociation constant) among histidine kinase receptor members (Romanov, Lomin, & Schmulling, 2006). Although originally thought to be localized at the cell surface, it is now accepted that cytokinin perception by the receptor is occurring in the endoplasmic reticulum and the membrane-bound receptor then relays its phosphorylation signal to the nucleus and activates or inhibits responsive genes leading to the regulation of cell division (Lomin, Yonekura-Sakakibara, Romanov, & Sakakibara, 2011; Wulfetange et al., 2011).

s0020 2.2 Auxin

p0020 After new cells are produced at the meristem, they start to expand and sometimes differentiate, which promotes organ growth and development. Many growth changes during plant body development are mediated by asymmetrical cell expansion rates across the organs. Early descriptions of experiments performed by Charles Darwin led him to postulate that aerial parts of plants bent toward light, a phenomenon known as phototropism (Darwin & Darwin, 1880). Along with geotropism and other asymmetrical growth responses, tropism is thought to be mediated by unequal rates of auxin transport down the different sides of the organ, in this case, promoting cell

elongation in the shaded side of a shoot and inhibiting cell elongation in the irradiated side of the shoot. This results in the shoot bending toward the direction of the light source. Plant sensitivity to auxin is context dependent; root growth is inhibited at 100 nM IAA, a naturally occurring auxin, and shoot growth is promoted at the same concentration when exogenously applied.

p0025 Because cell expansion is fundamental to plant organ growth and development, auxin is involved in many aspects of plant life, including flower development, lateral root initiation, and embryogenesis. Forward genetic screens of Arabidopsis mutants to isolate seedlings resistant to an auxin transport inhibitor drug identified an F-box motif protein, transport inhibitor response 1 (TIR1) (Ruegger et al., 1998). Its sequence similarity to a component of E3 ubiquitin ligase complex subsequently revealed that auxin and the receptor complex are subject to movement into the proteasome and thus, targeted for degradation (Gray, Kepinski, Rouse, Leyser, & Estelle, 2001). Auxin-TIR1, a ligand–receptor pair forms a protein complex with transcriptional repressors, Aux/IAA protein family and a cofactor, inositol hexakisphosphate (InsP6), and this association causes a degradation of the repressor proteins, resulting in increased expression of auxin responsive genes. The *tir1* receptor mutant is also insensitive to auxin or its synthetic analogs such as 2,4-D (2,4-dichlorophenoxyacetic acid), because the receptor mutant cells are not able to respond a higher level of auxin accumulated in the cells when its transport out of the bottom of each cell and down the stem is inhibited.

p0030 Arabidopsis mutants which lack four members of TIR1 auxin receptor genes show severely impaired growth and insensitivity to exogenous applied auxin (Dharmasiri et al., 2005). Consistent with this, the induction of an auxin responsive marker is completely absent in the roots of a triple receptor mutant (Scheitz, Luthen, & Schenck, 2013). However, the shoot of the quadruple mutant is still capable of responding to auxin-induced growth stimulation (Schenck, Christian, Jones, & Luthen, 2010). Since there are six members of TIR F-box receptor family and 29 Aux/IAA coreceptor proteins in the Arabidopsis genome, combinatorial interaction of F-box receptor and coreceptor proteins likely account for differential sensitivities of roots and shoots to exogenously applied auxin (Calderon Villalobos et al., 2012). A membrane-bound receptor kinase system is also implicated in auxin perception (Xu et al., 2014), but the structural and regulatory basis for this function has yet to be clearly established.

s0025 2.3 Brassinosteroid

p0035 The cell elongation rate clearly influences overall plant height since cell division is restricted to a small portion of the shoot and roots. Some Arabidopsis mutants that show severe dwarfism due to reduced stem length were found that were caused by the reduced production of brassinosteroids including brassinolide (Choe et al., 1999; Noguchi et al., 1999). This compound is a steroid that was initially isolated from *Brassica napus* pollen and is capable of inducing tissue enlargement (Grove et al., 1979). A collection of Arabidopsis mutant alleles responsible to brassinosteroid-insensitive phenotype were isolated by genetic screens (Clouse, Langford, & McMorris, 1996; Li & Chory, 1997). BRI1 (Brassinosteroid-insensitive 1) encodes a leucine-rich repeat receptor-like protein kinase that was shown to interact with brassinosteroid (Wang, Seto, Fujioka, Yoshida, & Chory, 2001). BRI1 uses a coreceptor BAK1 (BRI1-associated kinase 1) which involves trans-phosphorylation of the two receptor kinases at the plasma membrane (Nam & Li, 2002; Russinova et al., 2004; Sun et al., 2013; Wang et al., 2008). However, brassinosteroid-induced phosphorylation of BRI1 reported to date is occurring in the time scale of 60–120 min after treatment with the ligand, which appears to be much slower than the typical ligand-regulated phosphorylation responses reported with other receptor kinases such as EGFR whose phosphorylation by EGF treatment increases within 1 min and starts decreasing after 5–10 min (Curran, Zhang, Ma, Sarkaria, & White, 2015; Oh, Wang, Clouse, & Huber, 2012; Olsen et al., 2006; Tang et al., 2008; Wang et al., 2005).

p0040 Consistent with genetic evidence showing that many critical amino-acid residues for BRI1 function are localized within the extracellular island domain, structural analyses demonstrated that brassinolide interacts with the binding pocket found in this island domain (Hothorn et al., 2011; She et al., 2011). This BRI1 receptor kinase signaling for plant steroid signal is perceived much different from animal steroid hormone receptor system, which takes place within the nucleus and directly regulates gene expression (Hall, Couse, & Korach, 2001; Shao & Brown, 2004). Despite the clear importance of the BRI1 receptor derived from genetic ablation, there is no clear evidence that brassinosteroids are differentially transported or metabolized during any aspect of plant life (Symons, Ross, Jager, & Reid, 2008). In this characteristic, brassinosteroids may be different from other plant hormones, and perhaps acting more like a cofactor than a hormone,

which perhaps is a reflection of the fact that the plasma membrane is the most sterol rich membrane in the cell. Moreover, unlike other membrane-bound receptors which respond to their ligands, BRI localization, turnover, and ubiquitination are shown to be independent of brassinolide (Geldner, Hyman, Wang, Schumacher, & Chory, 2007; Martins et al., 2015).

s0030 2.4 Abscisic Acid

p0045 A critical role for protein phosphorylation in regulating plant growth is clear in the case of the ABA receptor system. ABA, first identified from abscising (a form of cell localized cell death) cotton tissue and sycamore trees, is known to be involved in a broad range of stress responses, water usage regulation, and seed desiccation/dormancy processes (Finkelstein, 2013). Although there were several earlier reports of genes encoding proteins that had characteristics of an ABA receptor, none of these proteins proved to be the actual receptor(s). The great difficulty in isolating putative ABA receptors via genetic screens hinted that ABA receptors might be members of a large redundant gene family that individually mask the phenotype, thus failing to identify receptor mutants from screens of single gene mutations. The use of a chemical genetic screen, involving small chemicals termed pyrobactins acting as a tissue-specific analog of ABA, enabled the isolation of the first bona fide ABA receptor mutants. A family of soluble ABA receptor proteins, the pyrobactins, PYRs, binds to ABA in a stereospecific manner and the bound form of PYRs can physically interact with and inhibit a specific group of protein phosphatases, PP2C. This inhibition causes the increase of phosphorylation in the activation domain of specific protein kinases, the SnRKs (Ma et al., 2009; Park et al., 2009). SnRK activation triggers phosphorylation of other target proteins including ABA-responsive transcription factors (Umezawa et al., 2013). A higher order of *pyr* receptor mutants, quadruple- or sextuple mutants, were produced and revealed relevant phenotypes (Gonzalez-Guzman et al., 2012). Consistent with ABA roles in plants as a stress hormone, *pyr* receptor mutants exhibit defects in the regulation of stomatal pore closing in response to drought stress. The use of a modified version of the PYR receptors also rendered plants able to withstand drought stress, implicating a powerful new means of modulating plant physiology via editing hormone receptor function (Park et al., 2015).

s0035 2.5 Ethylene

p0050 During the seedling growth phase at which a root and shoot first emerge and extend from a seed, growth and development are carefully coordinated. A seed deposited in soil experiences darkness and thus undergoes etiolation: elongated hypocotyl (the first stem of a seedling), shorter root, small and pale leaves, and folded cotyledonary leaves (the first leaves of a plant, which were stored in the embryo). As a plant develops, the cotyledonary leaves open, an apical hook unfolds, hypocotyl elongation ceases, and the root elongates. During this transition, a gas known as ethylene plays important roles. Treatment of germinating seedlings with ethylene causes the well known "triple response," i.e., a curved apical hook, short and thick hypocotyl, and reduced root elongation. Genetic screens identified a mutant which does not show the triple response and the gene encoding this mutation was identified as an ethylene receptor, ETR1, a protein distantly related to the receptor protein for cytokinin (Bleecker, Estelle, Somerville, & Kende, 1988; Chang, Kwok, Bleecker, & Meyerowitz, 1993) (see the above). Heterologous expression of the ETR1 receptor protein in yeast confirmed that ethylene directly binds to ETR1 (Schaller & Bleecker, 1995).

p0055 Binding of ethylene to ETR1 at the endoplasmic reticulum causes inactivation of both the receptor and its downstream protein, CTR1 (constitutive triple response), a Raf-like serine threonine kinase. These events then signal to EIN2 (ethylene insensitive 2), a transmembrane protein. The C-terminal end of EIN2 is then cleaved and translocated from the ER to the nucleus to induce activation of the EIN3 family of ethylene responsive transcription factors (Qiao et al., 2012).

s0040 2.6 Gibberellin

p0060 Stem elongation is regulated by complex signaling pathways involving various hormones, including auxin, brassinolide, and gibberellin. A diterpenoid compound, gibberellin, was first discovered from a fungus due to its effect on rice causing elongated stem. This compound was later shown to be an endogenously synthesized hormone that regulates stem cell elongation. Gibberellin acts through its nucleus-localized receptor, GID1 (Gibberellin-Insensitive Dwarf1), and like auxin, this binding induces degradation of transcriptional repressor proteins, DELLA which have a characteristic conserved motif Asp-Glu-Leu-Leu-Ala, at the amino terminus (Ueguchi-Tanaka et al., 2005). Mutations in gene sequences

involved in gibberellin signaling or biosynthesis cause the production of dwarf plants, due to a reduction in stem cell length. In these mutants with shorter stems, energy from photosynthesis is diverted to grain production instead of to stem growth and this has contributed to increased crop yield responsible for the Green Revolution, for which the Nobel Peace Prize was awarded in 1970, and subsequent research demonstrated that the mutations were localized in gibberellin synthesis and receptor signaling components.

s0045 2.7 Jasmonic Acid

p0065 Previously, plant growth and development were thought to be regulated by five classical hormones, auxin, cytokinin, gibberellin, ethylene, and ABA (see the above). More recent studies now are acknowledging other small molecules, including jasmonic acid and strigolactones (see later) as either growth regulatory substances or plant hormones involved in cell-to-cell communication. Jasmonic acid, a volatile chemical derived from linolenic acid, is involved in senescence (biological aging), defense responses, and growth inhibition. The jasmonic acid biosynthesis via cyclic reaction from this fatty acid is often compared with the mammalian compound, prostaglandin, a hormone-like compound, which acts during inflammation and vasodilation (widening blood vessels), and is biosynthesized from arachidonic acid. Despite similarity in chemical structure, clearly jasmonic acid and prostaglandin share no similarities in their downstream biological response.

p0070 Jasmonic acid synthesis is induced by mechanostress and induces the expression of genes involved in defense responses. Methyl jasmonate, a volatile derivative, also diffuses into the air and induces defense responses in neighboring plants (Farmer & Ryan, 1990). Coronatine is a synthetic analog of jasmonic acid used to study jasmonic acid function and signaling in plants. A *coronatine insensitive 1* (*coi1*) mutant that was also resistant to jasmonate was shown to have a mutation in a gene encoding an F-box protein which shows a sequence similarity to TIR1, an auxin receptor (Sheard et al., 2010; Xie, Feys, James, Nieto-Rostro, & Turner, 1998). Three-dimensional analysis of the jasmonic acid receptor, COI1, revealed that COI1 can bind to a conjugated form of this hormone, jasmonate-isoleucine, a coreceptor JAZ protein, transcriptional repressors, and a cofactor, inositol pentakisphosphate (Sheard et al., 2010).

s0050 2.8 Strigolactone

p0075 Overall plant body shape at maturity is influenced by shoot branching patterns. Branch development is controlled by genetic and environmental factors, which integrate hormone transport and action, leading to the emergence and elongation of lateral branches. For example, maize (corn) and its ancestor, teosinte, are dramatically different in shoot branching patterns, presumably due to the genetic selection derived from domestication by humans over thousand years. Teosinte branched1 (TB1), encoding a transcription factor, suppresses the bud growth (Doebley, Stec, & Hubbard, 1997). An important role for TB1 as a key genetic component of branching pattern was supported by the identification of an Arabidopsis gene related to TB1. Thus, the teosinte branched 1-like 1 gene was identified and its knockout mutant exhibits a hyperbranching phenotype (Finlayson, 2007).

p0080 Characterization of bushy mutants of Arabidopsis revealed that they contain reduced concentrations of strigolactone, indicating that this may be a new type of plant hormone. Application of this compound to plants inhibits shoot branching (Umehara et al., 2008). A strigolactone-insensitive plant, d14 (Dwarf14), isolated from rice produces increased side shoots (tillers) (Arite et al., 2009). D14 of rice and its orthologs, AtD14 of Arabidopsis and *deceased apical dominance 2* (*dad2*) of petunia are strigolactone receptors, which are members of the α/β-hydrolase enzyme superfamily and distantly related to a gibberellin receptor, GID (Hamiaux et al., 2012). Interaction of strigolactone with the receptor causes hydrolysis of the ligand, which promotes the ligand–receptor complex interacting with MAX2, an F-box protein, leading to proteasome-mediated degradation of target proteins (Wang et al., 2013). The Arabidopsis mutant, *max2*, shows increased shoot branching and the gene encoding this protein is involved in the signaling of seed germination promoted by another lactone compound found in smoke, karrikin (Nelson et al., 2011; Stirnberg, van De Sande, & Leyser, 2002). Although it is well known that some plant seeds require smoke for germination and appear to be involved in the changes in floral species found within a forest after fires, there is no evidence for this compound derived from forest fire smoke to be naturally synthesized in planta to date.

p0085 In conclusion, the half dozen or so small molecules described earlier are critical growth regulators during plant growth and development. Until ~20 years ago, it was believed that plants use only small molecules for regulating growth and development. However, the presence of a large family of

orphan plasma membrane receptor-like kinase genes in the plant genome (but few or no G protein-coupled receptors), suggest that plant cells could be perceiving many undiscovered ligands at the plasma membrane via these kinases, perhaps to help coordinate growth and development during the many discrete steps of differentiation and function that occur at various parts and times in plant life.

s0055

3. PLASMA MEMBRANE AS THE SITE OF PEPTIDE LIGAND SENSING BY RECEPTORS

p0090 In animals, the plasma membrane sodium pump (Na^+/K^+-ATPase) provides the membrane potential and sodium gradient utilized by ion channels and cotransporters. In plants (and fungi), a plasma membrane proton pump plays a similar role in creating the primary electrochemical gradient that drives all of the channels and cotransporters. In this case, the gradient created by the pump is a much higher membrane potential (minus 250 mV resting potential) and a chemical gradient of protons. In fact, sodium is an unessential element in plants and plays no critical role in any specific plant process. Despite this important difference in fundamental energetics of the plasma membrane, changes in the cytoplasmic concentration of calcium are universal signaling events in both animals and plants. Mechanostimuli, neurotransmitters, and peptide growth factors all induce a cytoplasmic calcium transient as a second messenger, and this is often associated with phospholipid alterations and rapid changes in protein phosphorylation initiated at the plasma membrane. Although plants do not have neurons, they do exhibit rapid signal propagation via calcium waves at the organismal level in response to environmental stimuli such as abiotic stresses (Choi, Toyota, Kim, Hilleary, & Gilroy, 2014). Furthermore, the presence of many small open reading frame (ORF) genes with predicted secretion signal sequences in the genome clearly supports the idea that hormone-like peptides may play diverse physiological roles to activate intracellular signaling.

p0095 In order to biochemically identify such peptide ligands, we set up a high-throughput assay for endogenous peptides that induce a cytoplasmic calcium transient in Arabidopsis. Using a high-resolution reverse phase HPLC column, we fractionated all of the small proteins and peptides derived from a soluble extract and purified and sequenced a few micrograms of the peptide responsible for one of the major peaks of activity. The sequence of this peptide revealed that it was a homolog of RALF (rapid alkalinization factor) a

5000-Da peptide previously identified on the basis of its ability to cause alkalinization of the cellular medium upon bathing plant cells and predicted to interact with a receptor complex composed of 120 and 25 kDa proteins (Pearce, Moura, Stratmann, & Ryan, 2001b; Scheer, Pearce, & Ryan, 2005). In addition to its ability to induce cell wall alkalinization and cytoplasmic calcium transients, this peptide also suppresses cell elongation in roots when applied to plants. In order to obtain new information on the signaling pathway induced by RALF1, we performed a mass spectrometry-based quantitative analysis of the plasma membrane phosphoproteome in Arabidopsis seedlings. We observed that within 5 min of RALF treatment of Arabidopsis seedlings phosphorylation of a serine residue (S899) in the carboxy terminal regulatory tail of the Arabidopsis plasma membrane H^+-ATPase 2 (AHA2) was increased over fourfold without altering overall AHA2 protein level. In the same dataset of RALF-induced changes in the plasma membrane phosphoproteome, we identified and quantified ~600 phosphopeptides and the largest change (10- to 20-fold) in phosphopeptide abundance was observed in the FERONIA receptor kinase within two phosphopeptides located at the regulatory carboxy terminus. Based on this observation and by analogy to mammalian epidermal growth factor or insulin-induced increases in the phosphorylation of cognate tyrosine receptor kinases, we hypothesized that RALF acts through FERONIA to downregulate root growth. We tested this hypothesis using reverse genetics to study the effect of the absence of FERONIA on RALF sensitivity and also, via direct biochemical studies of RALF binding to FERONIA (Haruta, Sabat, Stecker, Minkoff, & Sussman, 2014). A recent study examining RALF-induced rapid FERONIA phosphorylation and ABA-induced FERONIA phosphorylation as a long-term response (i.e., 1–4 h incubation) suggests that FERONIA is also involved in abiotic stress responses (Chen et al., 2016).

s0060

4. FERONIA AND ITS GENE FAMILY IN PLANT GROWTH AND DEVELOPMENT

p0100 The FERONIA receptor kinase was first identified with a genetic mutant, *feronia* (or Sirene) in which the pollen tube does not stop its elongating growth when it reaches the ovule of female gamete, thus causing pollen tube overgrowth, reduced fertility, and semisterile phenotype (Escobar-Restrepo et al., 2007; Huck, Moore, Federer, & Grossniklaus, 2003; Rotman,

Gourgues, Guitton, Faure, & Berger, 2008; Rotman et al., 2003). The gene was named after the fertility Goddess, FERONIA from an Etruscan legend of ancient Italy. FERONIA is located at the surface of the synergid cells supporting the egg cells within the ovule and was thought to recognize an unknown signal molecule, presumably localized at the pollen tip. Once FERONIA interacts with signals from the pollen tube, the synergid cells undergo programmed cell death and the pollen tube bursts to release the sperm nuclei, resulting in gamete fusion. The extracellular domain of FERONIA is distantly related to malectin, a protein found in the endoplasmic reticulum of mammalian cells. Malectin is involved in protein glycosylation and interacts with a diglucose moiety (Schallus, Feher, Sternberg, Rybin, & Muhle-Goll, 2010; Schallus et al., 2008). Because of a small amount of sequence similarity of the ectodomain of FERONIA to mammalian malectin, it was speculated that FERONIA ectodomain may interact with sugar or other carbohydrate-like molecules present in the cell wall (Boisson-Dernier, Kessler, & Grossniklaus, 2011).

p0105 Although FERONIA was first discovered as a female determinant of pollen-egg recognition, the FERONIA gene is widely expressed in all vegetative tissues and developmental stages except in pollen, suggesting that its role is not limited to ovule fertilization (Lindner, Muller, Boisson-Dernier, & Grossniklaus, 2012). A role of FERONIA as a plasma membrane component involved in initiating cellular signaling in other cell types was also evident from experiments showing its interaction with guanine nucleotide exchange factors (GEFs), ROP-GEF, during root hair development (Duan, Kita, Li, Cheung, & Wu, 2010). The involvement of FERONIA in cell growth and/or polarized cell growth was observed in *feronia* mutants which display collapsed, burst, or arrested root hairs. The root hair is a single cell extended from the epidermal cell in roots and thus is often considered to share common "tip growth" regulatory mechanisms, including calcium ion, reactive oxygen species, GTP exchange reaction, with pollen tube cell elongation. Interestingly, a glycosylphosphatidylinositol (GPI) anchored protein, LORELEI-LIKE GPI ANCHORED protein 1 (LLG1), whose homologous gene LORELEI functions in ovule fertilization in a similar manner to FERONIA, is also reported to be required for root hair development. The knockout plant containing a null mutation of *llg1* showed defective root hair development resembling to *feronia* mutant. A subsequent biochemical experiment showed that LLG1 is a component of RALF1-FERONIA signaling and was demonstrated to interact with RALF1 and its receptor protein kinase, FERONIA (Li et al., 2015).

p0110 FERONIA belongs to a family of 16 receptor-like kinases known as CrRLKs (*Catharanthus roseus* receptor-like kinase), since the first gene encoding a member of this receptor kinase family was discovered from cell cultures of *C. roseus* (Madagascar periwinkle) (Schulze-Muth, Irmler, Schroder, & Schroder, 1996). A cDNA encoding this protein was expressed as a truncated version containing a protein kinase domain and it was demonstrated that the gene encodes an active protein kinase which autophosphorylates at serine and threonine residues within the protein. Another member of the CrRLK family, THESEUS, was reported in a study of suppressor screens of a cellulose synthase (*procuste1*, *prc1*) mutant, which showed defective hypocotyl elongation under dark conditions (Hematy et al., 2007). Recessive mutations in *theseus* can suppress a shorter hypocotyl phenotype seen with a cellulose synthase, *prc1* mutant, but this mutation does not affect the hypocotyl of wild-type plants. Thus, it was proposed that THESEUS is a sensor for cell wall integrity, although the molecular or biochemical definition for "cell wall integrity" remains unclear. One may also speculate that the effect of *theseus* mutation is visible only when the constraint by a rigid cell wall is reduced in the cellulose synthase mutant. If THESEUS is a negative regulator of cell expansion as is the case of FERONIA, all of the observations also fit a model in which THESEUS is normally restricting cell expansion at the plasma membrane and its knockout phenotype cannot be detected when the cell wall is restraining cell elongation, in the wild-type plant.

p0115 The idea that CrRLK is involved in sensing changes in cell wall composition hinted that FERONIA may be also playing a role during mechanosensing signal transduction. Plants naturally experience mechanical stresses from the environments or load-bearing of their own tissue weights as well as by touch from insects and fixed structures (e.g., peas wrapping their tendrils around a pole), and thus, sense changes in tensile strain by rapid changes in calcium concentration in the cytoplasm and pH in the cell wall. During this process, *feronia* mutants show impaired calcium or proton ion fluxes and reduced frequencies of penetrating into a high-density agar (Shih, Miller, Dai, Spalding, & Monshausen, 2014). In addition to the rapid changes in ion signaling, mechanical stimuli induce protein phosphorylation (Piotrowski, Liss, & Weiler, 1996). The FERONIA receptor kinase, which was shown to be an active kinase when expressed as a kinase domain in *Escherichia coli*, may be involved in mechanosensing with its kinase function since a kinase-negative mutant version of FERONIA showed a different activity compared with wild-type FERONIA. This is an intriguing result

since the role of FERONIA in the ovule fertilization does not seem to rely on its kinase activity (Kessler, Lindner, Jones, & Grossniklaus, 2015); instead, a cytoplasmic protein kinase may be involved in its phosphorylation signaling downstream of the CrRLKs (Boisson-Dernier, Franck, Lituiev, & Grossniklaus, 2015). It remains to be examined whether a kinase-negative mutation of this cytoplasmic kinase affects its biological function.

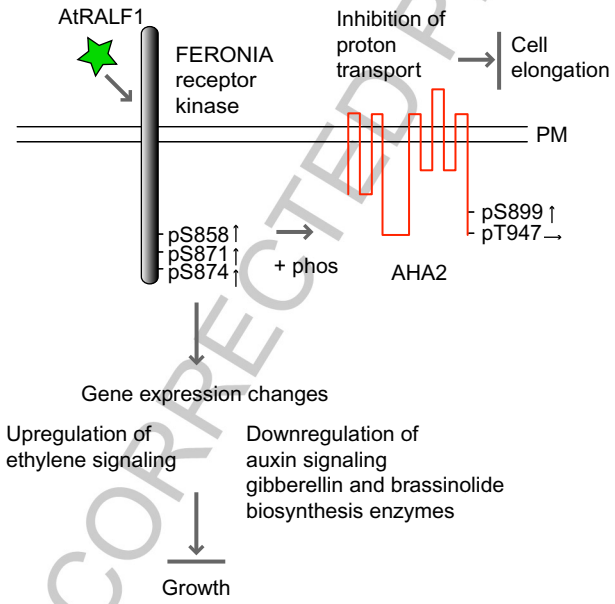
p0120 Since the plasma membrane H^+-ATPase is an essential protein involved in all aspects of plant physiology (Haruta et al., 2010; Haruta & Sussman, 2012), RALF and FERONIA acting directly through changes in the catalytic activity of the pump may contribute to the pleiotropic mutant phenotypes observed with *feronia* knockout plants. Yu et al. observed that growth of the *feronia* mutant is hypersensitive to the presence of ABA (Yu et al., 2012). A genetic mutant screen of ethylene hypersensitive plants also identified *feronia* mutants (Deslauriers & Larsen, 2010). Although there is no causal link established, FERONIA has also been reported to function by directly interacting with a metabolic enzyme, glyceraldehyde-3-phosphate dehydrogenase to inhibit starch accumulation (Yang et al., 2015), as well as interacting with *S*-adenosylmethionine synthetase to suppresses ethylene production (Mao et al., 2015).

p0125 Two other members of the CrRLK family, ANXUR1 and ANXUR2, are collectively required for pollen tube elongation as a homozygous double knockout for the two genes caused lethality due to pollen germination and elongation defects (Boisson-Dernier et al., 2009; Miyazaki et al., 2009). The mutant pollen grains with the haplotype *anx1/anx2* can be formed, but the pollen tube ruptures before they elongate. From the mutants' phenotype, it is possible that normal function of the ANXUR receptor kinase is restricting pollen tube elongation. When restriction is removed in the double mutants, a pollen tube bursts. It remains to be tested whether FERONIA and ANXUR share the same ligands during fertilization functions.

s0065 5. ORGAN SIZE AND GROWTH ARE DETERMINED BY STIMULATION AND INHIBITION BY SIGNALING PATHWAYS IN PLANTS AND ANIMALS

p0130 In the first several days after seed imbibition starts, storage nutrients support organ expansion until a plant enters a developmental stage in which it is capable of photosynthesis. In this transition, changes in hormone metabolism occur to regulate growth and to optimally respond to the environment (Bewley, 1997; Bhalerao et al., 2002; Fait et al., 2006). Young seedlings are

not photosynthetically active and their growth relies on the preserved nutrients in the seed endosperm. Thus, during the seedling stage at which plants establish photosynthetic capability, plants have sensitive sensor mechanisms to perceive light intensity and to modify growth and development. A seedling that is growing faster than necessary under a condition in which there is not enough light available, would result in deleterious consequences by using up the reserved nutrients. Plants have growth regulatory mechanisms to actively "ratchet" down the growth rate and reduce the rate of cell elongation. In the authors' previously described discovery of RALF-FERONIA, a peptide ligand and receptor kinase pair, acting as a negative regulator of cell elongation, fit into this model (Haruta et al., 2014). The primary action of this peptide–receptor signaling is to inactivate plasma membrane H^+-ATPase that provides energy to the plasma membrane, so that they can import the nutrients required for cell growth (Fig. 1).



f0005 **Fig. 1** Model for rapid RALF-FERONIA-regulated cell growth. RALF binding to the ectodomain of FERONIA receptor kinase induces a rapid phosphorylation at regulatory Ser residues at the carboxy terminus. RALF-induced activation of FERONIA either directly or indirectly increases the level of phosphorylation of AHA2 at Ser899. This results in inhibition of proton efflux, a rise in cell wall pH and suppression of cell growth. In the long term (e.g., 30 min), RALF modulates the expression of genes involved in cell elongation. Transcripts for genes encoding proteins involved in growth stimulatory mechanisms are reduced by RALF action.

p0135 Signaling mechanisms by which cells actively suppress growth and development are also essential in mammalian systems. Organismal growth in animals is mediated by cell proliferation, growth, and migration. Failure in appropriate regulation of such inhibitory mechanisms causes organ overgrowth or cancer development. For example, loss-of-function mutations in Hippo serine/threonine kinase of fruit fly result in organ overgrowth that disrupt eye and wing pattern development (Harvey, Pfleger, & Hariharan, 2003). Another example is kinase-mediated inhibition of cell migration. A deletion of ERK8 (extracellular signal-regulated kinase, also known as MAPK15, ERK7) causes twofold faster cell migration in Hela cells (Chia, Tham, Gill, Bard-Chapeau, & Bard, 2014). Moreover, ERK8 is known to induce degradation of the estrogen receptor and a loss of its expression is correlated with increased progression of breast cancer (Henrich et al., 2003).

s0070 6. RALF FAMILY AND FUNCTION IN PLANT GROWTH AND DEVELOPMENT

p0140 With the completion of many plant and microorganism genomes, bioinformatic analyses identified a family of RALF-like sequences throughout the plant kingdom. In the Arabidopsis genome, there are 34 RALF-like sequences. The widespread expression profiles of RALF-like sequences were also evident from some earlier mRNA sequencing analyses obtained by the expression sequence tags approach from the reproductive tissue of tomato plants (Germain, Chevalier, Caron, & Matton, 2005). A loss-of-function study of a tomato RALF (*Solanum chacoense* RALF3, ScRALF3) revealed that silencing this gene caused a reduction in seed production due to defective development of the embryo sac (Chevalier, Loubert-Hudon, & Matton, 2013). Female gamete development, referred to as megagametogenesis, involves a series of meiotic cell divisions, cell death, and mitosis to produce eight nuclei. Since our Arabidopsis RALF1/FERONIA peptide hormone receptor kinase cognate pair model predicts that RALF1 suppresses cell growth by inactivation of AHA2, the phenotype seen with ScRALF3-silenced tomato plants is consistent with this hypothesis that growth termination is a necessary process during female gametogenesis.

p0145 The involvement of RALF peptides in pollen tube elongation was examined by an in vitro assay using a tomato system. Application of SlRALF

(*Solanum lycopersicum* RALF) inhibits pollen tube elongation, but does not affect pollen grain hydration, with sequence specific and pH-dependent effects (Covey et al., 2010). Additional evidence that RALF-like peptides play roles in pollen function was observed in a transcriptome study via RNA sequencing. RALF-like 8 and 9 transcripts are among those most abundant and rank at 7th and 11th, respectively, among the 33,614 genes in pollen (Loraine, McCormick, Estrada, Patel, & Qin, 2013). RALF-like 8, which is expressed at a very low level in seedlings in the normal growth condition, was shown to be transcriptionally induced in Arabidopsis seedlings when exposed to consecutive stresses by a challenge with parasitic-nematode infestation, followed by water-deficit condition (Atkinson, Lilley, & Urwin, 2013). Overexpression of RALF-like 8 gene under a constitutive promoter resulted in shorter roots with excessive root hair production. The RALF-like 8 overexpressor is also more susceptible to drought stress. The effects of RALF peptide overexpression were also reported with RALF1 and RALF-like 23, both resulting in semi-dwarf phenotypes (Matos, Fiori, Silva-Filho, & Moura, 2008; Srivastava, Liu, Guo, Yin, & Howell, 2009). The dwarfism seen with RALF1 overexpressors was dependent on processing of the prepro-RALF precursors into the mature peptides since mutating the predicted protease recognition site of the RALF precursor abolished the dwarf phenotype.

p0150 Whereas the phenotypes observed with gene overexpression experiments require caution in interpretation due to possible indirect effects, gene knockout or silencing experiments provide more reliable clues on biological function of RALF peptide. Wu et al. reported that tobacco appears to have a smaller RALF gene family than Arabidopsis and that tobacco plants in which RALF expression is silenced showed reduced root hair development and a longer root (Wu et al., 2007).

s0075 7. RALF-LIKE PEPTIDES ARE NOT ONLY PRODUCED BY PLANTS BUT ALSO BY PATHOGENIC MICROBES

p0155 Plants in nature are continuously exposed to microbes, some of which are beneficial for normal growth and development (symbiosis) or some that are not (pathogenic infection). Both plants and symbiotic or pathogenic microbes secrete signaling molecules. Recent studies have identified genes encoding RALF-like peptides in the genome of a pathogenic fungus, *Fusarium oxysporum* (Masachis et al., 2016). The fungal-derived RALF peptide (f-RALF) was shown to act in a similar manner to endogenous plant RALF

peptide and was found to be capable of inducing extracellular alkalinization in tomato suspension cells and inhibition root elongation in tomato seedlings. More importantly, a *feronia* knockout plant which is insensitive to growth inhibition by Arabidopsis RALF1 is also insensitive to f-RALF peptide. Based on these observations, it was proposed that the fungus is suppressing plant cell growth to increase infection efficiency. In agreement with this idea, the *feronia* mutant, which does not respond to fungal RALF, is resistant to the pathogenic infection. Also consistent with this model is the observation that a deletion mutant of *F. oxysporum*, which lacks a gene encoding the f-RALF peptide infected tomato plants with a much reduced efficiency compared with the wild-type *F. oxysporum* strain.

p0160 Thynne et al. also identified RALF-like sequences in the genomes of 26 phytopathogenic fungi (Thynne et al., 2016). A RALF-like peptide, RALF-B from *F. oxysporum* f. sp. *lycopersici*, inhibits the growth of tomato seedlings. However, in this study, the authors suggested that the RALF peptide from *F. oxysporum* can influence pathogen virulence in only limited conditions, e.g., when plants were grown in media with reduced levels of nutrients.

s0080

8. OTHER ENDOGENOUS HORMONE-LIKE PEPTIDES AND THEIR RECEPTOR PAIRS FOR PLANT GROWTH, DEVELOPMENT, AND PHYSIOLOGY

p0165 Discovery of the first peptide acting as a hormone-like signal in plants dates back to 1991 when an 18-amino-acid long peptide, systemin was biochemically isolated as a factor capable of inducing a proteinase inhibitor protein from tomato leaves (Pearce, Strydom, Johnson, & Ryan, 1991). This peptide was reported to interact with a 160-kDa receptor protein at the plasma membrane and induced the downstream signals resulting in the expression of an array of defense-related proteins (Scheer & Ryan, 1999). This study opened up a paradigm of plant peptide signals, which are now perceived as a critical part of plant signaling.

p0170 Plant cells have sensitive mechanisms for responding to extracellular signal molecules. Changes in transmembrane ion fluxes and protein phosphorylation are observed very rapidly upon cellular recognition of signal compounds (Boller, 1995). Peptide-based signal molecules including systemin can elicit extracellular alkalinization of cultured cells (Felix & Boller, 1995). Thus, monitoring pH changes in the medium of cultured cells has been used as an assay to seek signal- or elicitor-like molecules which

activate cellular signaling for the activation of defense responses or growth regulation at the plasma membrane (Felix, Duran, Volko, & Boller, 1999; Kunze et al., 2004). A 23-amino-acid peptide, AtPep1 (*Arabidopsis thaliana* elicitor peptide1) was isolated using this alkalinization assay and shown to induce pathogen defense in Arabidopsis through an leucine-rich repeat (LRR) receptor-like kinase PEPR1, which forms a heterodimer with the BAK1 receptor kinase (Huffaker, Pearce, & Ryan, 2006; Tang et al., 2015; Yamaguchi, Pearce, & Ryan, 2006). The AtPep1 sequence shows limited sequence conservation when compared with maize peptides, possibly suggesting that this peptide–receptor pair may be functioning in a species-specific manner (Huffaker, Dafoe, & Schmelz, 2011; Lori et al., 2015).

p0175 As is the case of mammalian peptide growth factors or hormones, post-translational modification of peptides appears to be a common mechanism in plants. Phytosulfokine, a sulfated penta peptide isolated from asparagus, which can induce cell proliferation in cultured cells, is produced as a precursor protein and processed to a small biologically active peptide (Matsubayashi & Sakagami, 1996). A receptor for phytosulfokine, PSKR1 was identified via photoaffinity cross-linking and purification, followed by mass spectrometry sequencing. This peptide is recognized by a cell-surface receptor, LRR receptor-like kinase whose knockout caused shorter roots and overexpression caused longer roots and enhanced proliferation of cells (Matsubayashi, Ogawa, Kihara, Niwa, & Sakagami, 2006; Matsubayashi, Ogawa, Morita, & Sakagami, 2002). A crystal structural study revealed that PSK ligand binds to the PSKR1 receptor, which causes allosteric activation of the receptor and allows the ligand–receptor complex to interact with coreceptors known as somatic embryogenesis receptor-like kinases (SERKs) (Wang et al., 2015). An additional search for sulfated peptides resulted in the isolation of an 18-amino-acid tyrosine-sulfated glycopeptide, PSY1 from the media of Arabidopsis cell culture (Amano, Tsubouchi, Shinohara, Ogawa, & Matsubayashi, 2007). PSY1 stimulates root growth through a family of PSKR1, which interacts with and activates a plasma membrane H+-ATPase (Fuglsang et al., 2014). Posttranslational modification of bioactive peptides was also reported with systemin-like peptides from the Solanaceae family. Those peptides contain hydroxylated proline residues and glycosylation composed of pentose units. Multiple peptides are produced from a single precursor preproprotein and bioactive peptides induce wound-responsive genes, which function in antiherbivory defense responses (Pearce, Moura, Stratmann, & Ryan, 2001a; Pearce & Ryan, 2003); however,

receptor proteins for those systemin or systemin-like peptides remain to be clearly identified.

p0180 Some peptide–receptor pairs have been identified via genetic studies and later confirmed to directly interact using molecular biology techniques such as coimmunoprecipitation or biochemical cross-linking assay. A pair of CLAVATA3 (CLV3) ligand and CLAVATA1 (CLV1) receptor kinases is required for the maintenance of meristem cells to balance cell proliferation and differentiation, since insertion or point mutants of those genes cause increased apical bud formation (Clark, Williams, & Meyerowitz, 1997; Fletcher, Brand, Running, Simon, & Meyerowitz, 1999). The presence of an active form of CLV3 peptide was identified by in situ MALDI mass spectrometry (Kondo et al., 2006). A mass spectrum for the 12-amino-acid peptide localized at the carboxy terminus of CLV3 precursor protein was detected from Arabidopsis seedlings overexpressing the precursor protein. CLV3 peptide detected in situ contained two hydroxylated proline residues, although the hydroxylation did not influence the biological activity of the peptide. Photoaffinity labeling and a binding assay of CLV3 peptide interacting with CLV1 receptor also showed that CLV1-related receptor kinases, BAM1, 2, and 3 (Barely Any Meristem) are coreceptors for the CLV1–3 complex (DeYoung et al., 2006; Shinohara & Matsubayashi, 2015).

p0185 The Arabidopsis genome contains 26 genes encoding CLE peptides (CLAVATA3/embryo-surrounding region related, ESR) (Cock & McCormick, 2001). The CLE family shows the secretory signal sequence at the amino terminus and ~14-amino-acid conserved peptide sequence at the carboxy terminus. Application of synthetic peptides corresponding to the carboxy termini of CLV3, CLE19, or CLE40 caused shorter and thinner roots and decreases in the number of root meristematic cells (Fiers et al., 2005). Through biochemical search and purification, CLE41/44 peptide, originally named "tracheary element differentiation inhibitory factor" was found to be a factor that suppresses differentiation of mesophyll cells (cells found within leaf) into tracheary element (conducting cells in the vascular tissue) (Ito et al., 2006). CLE-like sequences are also found in the nematode genome and the precursor protein for the CLE-like peptide from potato cyst nematode *Globodera rostochiensis* (GrCLE1) was correctly processed in plants (Guo, Ni, Denver, Wang, & Clark, 2011; Wang et al., 2010). GrCLE1 inhibits root growth at the similar peptide concentration range as the endogenous peptide, CLV3. Moreover, GrCLE1 binds to Arabidopsis CLV2, BAM1, and BAM2 receptor kinase proteins, supporting a model in which the parasitic nematode uses GrCLE1 to mimic CLV3 peptide action.

p0190 Another example of a peptide–receptor pair identified initially based on a correlation of genetic mutant phenotypes is IDA (inflorescence deficient in abscission) peptide ligand, which interacts with its receptor HAESA (Latin word meaning to stick to) kinase and induces organ abscission (Butenko et al., 2003; Jinn, Stone, & Walker, 2000). In both *ida* and *haesa* mutants, floral organs including petal and stamens remain attached throughout silique (seed pod) development due to the loss of activation of abscission signaling. IDA interaction with HAESA induces the recruitment of SERKs and results in transphosphorylation of the two receptor kinases (Meng et al., 2016). Agreeing with this biochemical model, triple mutants of *serks* also exhibit defective organ abscission. Crystal structural analyses demonstrated that IDA dodeca peptide binds to HAESA with a weak affinity and SERK1 stabilizes the ligand–receptor complex (Santiago et al., 2016).

p0195 The availability of many plant genome sequences and bioinformatics tools has facilitated the identification of peptide ligands pairing with their receptor proteins. Based on the hypothesis that extracellularly secreted peptide signals are found in the genes encoding precursor proteins, small ORF genes were computationally screened. With this approach, a novel 15-amino-acid peptide, CEP1 (C-terminally encoded peptide 1) was identified (Ohyama, Ogawa, & Matsubayashi, 2008). Photoaffinity labeling assays performed with CEP1 peptide identified two LRR receptor kinases, from an Arabidopsis receptor kinase expression library produced in tobacco cells (Tabata et al., 2014). CEP-encoding genes were shown to be induced by nitrogen starvation, and together with their cognate receptor kinases, this family of peptides senses nitrogen availability in plants and regulates lateral root development. Peptide ligands, for a receptor-like kinase protein, known for regulating developmental pathways that produce or suppress guard cell production were also identified via a bioinformatic screen searching for small ORF genes. Peptides, named EPIDERMAL PATTERNING FACTOR 1 (EPF1), were found to suppress stomatal development by interacting with ERECTA receptor kinase (Hara, Kajita, Torii, Bergmann, & Kakimoto, 2007). Interestingly, stomatal development is also positively regulated by ERECTA interaction with another peptide, stomagen (Sugano et al., 2010). A current model suggests that competitive binding of stomagen and EPF1 onto the ERECTA receptor kinase determines stomatal density and patterning (Lee et al., 2015). EPF1 peptide induces heteromerization of ERECTA and SERKs in planta and this association predicts that the two receptor kinases cause transphosphorylation of

the proteins (Meng et al., 2015). This biochemical result is consistent with the genetic evidence that *serk* quadruple mutant also showed increased stomatal production.

p0200 In addition to RALF and EPF1, other peptides with cysteine disulfide bonds are involved in other cell-to-cell communications. A peptide related to defensin, LURE acts as an ovule-secreted signal to attract the pollen tube. LURE peptide, first identified based on gene expression profiling, was heterogeneously produced from *E. coli* and its function of guiding the pollen tube to the ovule was demonstrated (Okuda et al., 2009). A family of RLKs that are required for sensing LURE peptide in the pollen tube was identified and some of them were shown to directly interact with the peptide (Takeuchi & Higashiyama, 2016; Wang et al., 2016). Another example for a peptide hormone with cysteine disulfide bonds is the peptide–receptor pair for pollen perception by stigma (tip of the female structure) in the Brassicaceae family including Arabidopsis. A genetic mapping study revealed that this self-incompatibility locus contained the ligand and receptor genes. The inability of self-fertilization in some species of the Brassicaceae family is mediated by a peptide ligand, S-locus cysteine-rich protein (SCR) interacting with its receptor, S-locus receptor kinase (SRK). Binding of an incompatible SCR to SRK prevents pollen grain hydration, and thus, highly polymorphic sequences of the ligands and receptors within the species contribute to a higher degree of outcrossing (Kachroo, Schopfer, Nasrallah, & Nasrallah, 2001; Schopfer, Nasrallah, & Nasrallah, 1999; Takayama et al., 2001).

s0085 9. CONCLUSION AND FUTURE PERSPECTIVES

p0205 The development and growth of plants are regulated via the action of various hormones mediating either stimulation or inhibition of cell growth. The availability of Arabidopsis genetic and genomic resources has contributed greatly to revealing how plant hormones are perceived and act. Use of novel new techniques including chemical genetics as well as functional phosphoproteomic screens have made it possible to reveal the receptor protein identities for some important hormones. As we learn more about the complexity of intersecting hormone signaling pathways, we realize the value of a systems approach that includes profiling many different biomolecules, including proteins and their posttranslational modifications. We now know that some phytohormone receptors (e.g., auxin, GA, and jasmonate) are

F-box proteins which act through the proteolysis of transcriptional repressors. The Arabidopsis genome carries ~700 F-box proteins, and moreover, more than 5% of the genome encodes the proteins involved in the ubiquitin proteasome system (Gagne, Downes, Shiu, Durski, & Vierstra, 2002; Smalle & Vierstra, 2004). It is possible that there are many more small molecule growth regulators yet to be identified. Similarly, ligands for several hundreds of membrane-bound receptor kinase proteins still remain to be identified. Finally, due to the transient nature of protein interactions and phosphorylated amino acids, the continued development of robust and high-throughput biochemical assays will be helpful in order to reveal yet to be known signaling components that lead to plant growth regulation.

REFERENCES

Amano, Y., Tsubouchi, H., Shinohara, H., Ogawa, M., & Matsubayashi, Y. (2007). Tyrosine-sulfated glycopeptide involved in cellular proliferation and expansion in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(46), 18333–18338.

Arite, T., Umehara, M., Ishikawa, S., Hanada, A., Maekawa, M., Yamaguchi, S., & Kyozuka, J. (2009). d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. *Plant & Cell Physiology*, *50*(8), 1416–1424.

Atkinson, N. J., Lilley, C. J., & Urwin, P. E. (2013). Identification of genes involved in the response of Arabidopsis to simultaneous biotic and abiotic stresses. *Plant Physiology*, *162*(4), 2028–2041.

Bewley, J. D. (1997). Seed germination and dormancy. *Plant Cell*, *9*(7), 1055–1066.

Bhalerao, R. P., Eklof, J., Ljung, K., Marchant, A., Bennett, M., & Sandberg, G. (2002). Shoot-derived auxin is essential for early lateral root emergence in Arabidopsis seedlings. *The Plant Journal*, *29*(3), 325–332.

Bleecker, A. B., Estelle, M. A., Somerville, C., & Kende, H. (1988). Insensitivity to ethylene conferred by a dominant mutation in Arabidopsis thaliana. *Science*, *241*(4869), 1086–1089.

Boisson-Dernier, A., Franck, C. M., Lituiev, D. S., & Grossniklaus, U. (2015). Receptor-like cytoplasmic kinase MARIS functions downstream of CrRLK1L-dependent signaling during tip growth. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(39), 12211–12216.

Boisson-Dernier, A., Kessler, S. A., & Grossniklaus, U. (2011). The walls have ears: The role of plant CrRLK1Ls in sensing and transducing extracellular signals. *Journal of Experimental Botany*, *62*(5), 1581–1591.

Boisson-Dernier, A., Roy, S., Kritsas, K., Grobei, M. A., Jaciubek, M., Schroeder, J. I., & Grossniklaus, U. (2009). Disruption of the pollen-expressed FERONIA homologs ANXUR1 and ANXUR2 triggers pollen tube discharge. *Development*, *136*(19), 3279–3288.

Boller, T. (1995). Chemoperception of microbial signals in plant cells. *Annual Review of Plant Physiology and Plant Molecular Biology*, *46*, 189–214.

Butenko, M. A., Patterson, S. E., Grini, P. E., Stenvik, G. E., Amundsen, S. S., Mandal, A., & Aalen, R. B. (2003). Inflorescence deficient in abscission controls floral organ abscission in Arabidopsis and identifies a novel family of putative ligands in plants. *Plant Cell*, *15*(10), 2296–2307.

Calderon Villalobos, L. I., Lee, S., De Oliveira, C., Ivetac, A., Brandt, W., Armitage, L., … Estelle, M. (2012). A combinatorial TIR1/AFB-Aux/IAA co-receptor system for differential sensing of auxin. *Nature Chemical Biology*, *8*(5), 477–485.

Chang, C., Kwok, S. F., Bleecker, A. B., & Meyerowitz, E. M. (1993). Arabidopsis ethylene-response gene ETR1: Similarity of product to two-component regulators. *Science*, *262*(5133), 539–544.

Chen, J., Yu, F., Liu, Y., Du, C., Li, X., Zhu, S., … Luan, S. (2016). FERONIA interacts with ABI2-type phosphatases to facilitate signaling cross-talk between abscisic acid and RALF peptide in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(37), E5519–E5527.

Chevalier, E., Loubert-Hudon, A., & Matton, D. P. (2013). ScRALF3, a secreted RALF-like peptide involved in cell-cell communication between the sporophyte and the female gametophyte in a solanaceous species. *The Plant Journal*, *73*(6), 1019–1033.

Chia, J., Tham, K. M., Gill, D. J., Bard-Chapeau, E. A., & Bard, F. A. (2014). ERK8 is a negative regulator of O-GalNAc glycosylation and cell migration. *eLife*, *3*, e01828.

Choe, S., Dilkes, B. P., Gregory, B. D., Ross, A. S., Yuan, H., Noguchi, T., … Feldmann, K. A. (1999). The Arabidopsis dwarf1 mutant is defective in the conversion of 24-methylenecholesterol to campesterol in brassinosteroid biosynthesis. *Plant Physiology*, *119*(3), 897–907.

Choi, W. G., Toyota, M., Kim, S. H., Hilleary, R., & Gilroy, S. (2014). Salt stress-induced Ca^{2+} waves are associated with rapid, long-distance root-to-shoot signaling in plants. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(17), 6497–6502.

Clark, S. E., Williams, R. W., & Meyerowitz, E. M. (1997). The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis. *Cell*, *89*(4), 575–585.

Clouse, S. D., Langford, M., & McMorris, T. C. (1996). A brassinosteroid-insensitive mutant in Arabidopsis thaliana exhibits multiple defects in growth and development. *Plant Physiology*, *111*(3), 671–678.

Cock, J. M., & McCormick, S. (2001). A large family of genes that share homology with CLAVATA3. *Plant Physiology*, *126*(3), 939–942.

Covey, P. A., Subbaiah, C. C., Parsons, R. L., Pearce, G., Lay, F. T., Anderson, M. A., … Bedinger, P. A. (2010). A pollen-specific RALF from tomato that regulates pollen tube elongation. *Plant Physiology*, *153*(2), 703–715.

Curran, T. G., Zhang, Y., Ma, D. J., Sarkaria, J. N., & White, F. M. (2015). MARQUIS: A multiplex method for absolute quantification of peptides and posttranslational modifications. *Nature Communications*, *6*, 5924.

Darwin, C., & Darwin, F. (1880). *The power of movement in plants*. London: John Murray.

Deslauriers, S. D., & Larsen, P. B. (2010). FERONIA is a key modulator of brassinosteroid and ethylene responsiveness in Arabidopsis hypocotyls. *Molecular Plant*, *3*(3), 626–640.

DeYoung, B. J., Bickle, K. L., Schrage, K. J., Muskett, P., Patel, K., & Clark, S. E. (2006). The CLAVATA1-related BAM1, BAM2 and BAM3 receptor kinase-like proteins are required for meristem function in Arabidopsis. *The Plant Journal*, *45*(1), 1–16.

Dharmasiri, N., Dharmasiri, S., Weijers, D., Lechner, E., Yamada, M., Hobbie, L., … Estelle, M. (2005). Plant development is regulated by a family of auxin receptor F box proteins. *Developmental Cell*, *9*(1), 109–119.

Doebley, J., Stec, A., & Hubbard, L. (1997). The evolution of apical dominance in maize. *Nature*, *386*(6624), 485–488.

Duan, Q., Kita, D., Li, C., Cheung, A. Y., & Wu, H. M. (2010). FERONIA receptor-like kinase regulates RHO GTPase signaling of root hair development. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(41), 17821–17826.

Enders, T. A., & Strader, L. C. (2015). Auxin activity: Past, present, and future. *The American Journal of Botany*, *102*(2), 180–196.

Escobar-Restrepo, J. M., Huck, N., Kessler, S., Gagliardini, V., Gheyselinck, J., Yang, W. C., & Grossniklaus, U. (2007). The FERONIA receptor-like kinase mediates male-female interactions during pollen tube reception. *Science*, *317*(5838), 656–660.

Fait, A., Angelovici, R., Less, H., Ohad, I., Urbanczyk-Wochniak, E., Fernie, A. R., & Galili, G. (2006). Arabidopsis seed development and germination is associated with temporally distinct metabolic switches. *Plant Physiology*, *142*(3), 839–854.

Farmer, E. E., & Ryan, C. A. (1990). Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(19), 7713–7716.

Felix, G., & Boller, T. (1995). Systemin induces rapid ion fluxes and ethylene biosynthesis in Lycopersicon peruvianum cells. *Plant Journal*, 7, 381–389.

Felix, G., Duran, J. D., Volko, S., & Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *The Plant Journal*, *18*(3), 265–276.

Fiers, M., Golemiec, E., Xu, J., van der Geest, L., Heidstra, R., Stiekema, W., & Liu, C. M. (2005). The 14-amino acid CLV3, CLE19, and CLE40 peptides trigger consumption of the root meristem in Arabidopsis through a CLAVATA2-dependent pathway. *Plant Cell*, *17*(9), 2542–2553.

Finkelstein, R. (2013). Abscisic acid synthesis and response. *The Arabidopsis Book*, *11*, e0166.

Finlayson, S. A. (2007). Arabidopsis Teosinte Branched1-like 1 regulates axillary bud outgrowth and is homologous to monocot Teosinte Branched1. *Plant & Cell Physiology*, *48*(5), 667–677.

Fletcher, J. C., Brand, U., Running, M. P., Simon, R., & Meyerowitz, E. M. (1999). Signaling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems. *Science*, *283*(5409), 1911–1914.

Fuglsang, A. T., Kristensen, A., Cuin, T. A., Schulze, W. X., Persson, J., Thuesen, K. H., … Palmgren, M. G. (2014). Receptor kinase-mediated control of primary active proton pumping at the plasma membrane. *The Plant Journal*, *80*(6), 951–964.

Gagne, J. M., Downes, B. P., Shiu, S. H., Durski, A. M., & Vierstra, R. D. (2002). The F-box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(17), 11519–11524.

Geldner, N., Hyman, D. L., Wang, X., Schumacher, K., & Chory, J. (2007). Endosomal signaling of plant steroid receptor kinase BRI1. *Genes & Development*, *21*(13), 1598–1602.

Germain, H., Chevalier, E., Caron, S., & Matton, D. P. (2005). Characterization of five RALF-like genes from Solanum chacoense provides support for a developmental role in plants. *Planta*, *220*(3), 447–454.

Gonzalez-Guzman, M., Pizzio, G. A., Antoni, R., Vera-Sirera, F., Merilo, E., Bassel, G. W., … Rodriguez, P. L. (2012). Arabidopsis PYR/PYL/RCAR receptors play a major role in quantitative regulation of stomatal aperture and transcriptional response to abscisic acid. *Plant Cell*, *24*(6), 2483–2496.

Gray, W. M., Kepinski, S., Rouse, D., Leyser, O., & Estelle, M. (2001). Auxin regulates SCF(TIR1)-dependent degradation of AUX/IAA proteins. *Nature*, *414*(6861), 271–276.

Grove, M., Spencer, G., Rohwedder, W., Mandava, N., Worley, J., Warthen, J., … Cook, J. (1979). Brassinolide, a plant growth-promoting steroid isolated from Brassica napus pollen. *Nature*, *281*, 216–217.

Guo, Y., Ni, J., Denver, R., Wang, X., & Clark, S. E. (2011). Mechanisms of molecular mimicry of plant CLE peptide ligands by the parasitic nematode Globodera rostochiensis. *Plant Physiology*, *157*(1), 476–484.

Hall, J. M., Couse, J. F., & Korach, K. S. (2001). The multifaceted mechanisms of estradiol and estrogen receptor signaling. *The Journal of Biological Chemistry*, *276*(40), 36869–36872.

Hamiaux, C., Drummond, R. S., Janssen, B. J., Ledger, S. E., Cooney, J. M., Newcomb, R. D., & Snowden, K. C. (2012). DAD2 is an alpha/beta hydrolase likely to be involved in the perception of the plant branching hormone, strigolactone. *Current Biology*, *22*(21), 2032–2036.

Hara, K., Kajita, R., Torii, K. U., Bergmann, D. C., & Kakimoto, T. (2007). The secretory peptide gene EPF1 enforces the stomatal one-cell-spacing rule. *Genes & Development*, *21*(14), 1720–1725.

Haruta, M., Burch, H. L., Nelson, R. B., Barrett-Wilt, G., Kline, K. G., Mohsin, S. B., … Sussman, M. R. (2010). Molecular characterization of mutant Arabidopsis plants with reduced plasma membrane proton pump activity. *The Journal of Biological Chemistry*, *285*(23), 17918–17929.

Haruta, M., Sabat, G., Stecker, K., Minkoff, B. B., & Sussman, M. R. (2014). A peptide hormone and its receptor protein kinase regulate plant cell expansion. *Science*, *343*(6169), 408–411.

Haruta, M., & Sussman, M. R. (2012). The effect of a genetically reduced plasma membrane proton motive force on vegetative growth of Arabidopsis. *Plant Physiology*, *158*(3), 1158–1171.

Harvey, K. F., Pfleger, C. M., & Hariharan, I. K. (2003). The Drosophila Mst ortholog, hippo, restricts growth and cell proliferation and promotes apoptosis. *Cell*, *114*(4), 457–467.

Hematy, K., Sado, P. E., Van Tuinen, A., Rochange, S., Desnos, T., Balzergue, S., … Hofte, H. (2007). A receptor-like kinase mediates the response of Arabidopsis cells to the inhibition of cellulose synthesis. *Current Biology*, *17*(11), 922–931.

Henrich, L. M., Smith, J. A., Kitt, D., Errington, T. M., Nguyen, B., Traish, A. M., & Lannigan, D. A. (2003). Extracellular signal-regulated kinase 7, a regulator of hormone-dependent estrogen receptor destruction. *Molecular and Cellular Biology*, *23*(17), 5979–5988.

Hothorn, M., Belkhadir, Y., Dreux, M., Dabi, T., Noel, J. P., Wilson, I. A., & Chory, J. (2011). Structural basis of steroid hormone perception by the receptor kinase BRI1. *Nature*, *474*(7352), 467–471.

Huck, N., Moore, J. M., Federer, M., & Grossniklaus, U. (2003). The Arabidopsis mutant feronia disrupts the female gametophytic control of pollen tube reception. *Development*, *130*(10), 2149–2159.

Huffaker, A., Dafoe, N. J., & Schmelz, E. A. (2011). ZmPep1, an ortholog of Arabidopsis elicitor peptide 1, regulates maize innate immunity and enhances disease resistance. *Plant Physiology*, *155*(3), 1325–1338.

Huffaker, A., Pearce, G., & Ryan, C. A. (2006). An endogenous peptide signal in Arabidopsis activates components of the innate immune response. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(26), 10098–10103.

Inoue, T., Higuchi, M., Hashimoto, Y., Seki, M., Kobayashi, M., Kato, T., … Kakimoto, T. (2001). Identification of CRE1 as a cytokinin receptor from Arabidopsis. *Nature*, *409*(6823), 1060–1063.

Ito, Y., Nakanomyo, I., Motose, H., Iwamoto, K., Sawa, S., Dohmae, N., & Fukuda, H. (2006). Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science*, *313*(5788), 842–845.

Jinn, T. L., Stone, J. M., & Walker, J. C. (2000). HAESA, an Arabidopsis leucine-rich repeat receptor kinase, controls floral organ abscission. *Genes & Development*, *14*(1), 108–117.

Kachroo, A., Schopfer, C. R., Nasrallah, M. E., & Nasrallah, J. B. (2001). Allele-specific receptor-ligand interactions in Brassica self-incompatibility. *Science*, *293*(5536), 1824–1826.

Kessler, S. A., Lindner, H., Jones, D. S., & Grossniklaus, U. (2015). Functional analysis of related CrRLK1L receptor-like kinases in pollen tube reception. *EMBO Reports*, *16*(1), 107–115.

Kondo, T., Sawa, S., Kinoshita, A., Mizuno, S., Kakimoto, T., Fukuda, H., & Sakagami, Y. (2006). A plant peptide encoded by CLV3 identified by in situ MALDI-TOF MS analysis. *Science*, *313*(5788), 845–848.

Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T., & Felix, G. (2004). The N terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants. *Plant Cell*, *16*(12), 3496–3507.

Lee, J. S., Hnilova, M., Maes, M., Lin, Y. C., Putarjunan, A., Han, S. K., … Torii, K. U. (2015). Competitive binding of antagonistic peptides fine-tunes stomatal patterning. *Nature*, *522*(7557), 439–443.

Li, J., & Chory, J. (1997). A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell*, *90*(5), 929–938.

Li, C., Yeh, F. L., Cheung, A. Y., Duan, Q., Kita, D., Liu, M. C., … Wu, H. M. (2015). Glycosylphosphatidylinositol-anchored proteins as chaperones and co-receptors for FERONIA receptor kinase signaling in Arabidopsis. *eLife*, *4*, e06587.

Lindner, H., Muller, L. M., Boisson-Dernier, A., & Grossniklaus, U. (2012). CrRLK1L receptor-like kinases: Not just another brick in the wall. *Current Opinion in Plant Biology*, *15*(6), 659–669.

Lomin, S. N., Yonekura-Sakakibara, K., Romanov, G. A., & Sakakibara, H. (2011). Ligand-binding properties and subcellular localization of maize cytokinin receptors. *Journal of Experimental Botany*, *62*(14), 5149–5159.

Loraine, A. E., McCormick, S., Estrada, A., Patel, K., & Qin, P. (2013). RNA-seq of Arabidopsis pollen uncovers novel transcription and alternative splicing. *Plant Physiology*, *162*(2), 1092–1109.

Lori, M., van Verk, M. C., Hander, T., Schatowitz, H., Klauser, D., Flury, P., … Bartels, S. (2015). Evolutionary divergence of the plant elicitor peptides (Peps) and their receptors: Interfamily incompatibility of perception but compatibility of downstream signalling. *Journal of Experimental Botany*, *66*(17), 5315–5325.

Ma, Y., Szostkiewicz, I., Korte, A., Moes, D., Yang, Y., Christmann, A., & Grill, E. (2009). Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science*, *324*(5930), 1064–1068.

Mahonen, A. P., Higuchi, M., Tormakangas, K., Miyawaki, K., Pischke, M. S., Sussman, M. R., … Kakimoto, T. (2006). Cytokinins regulate a bidirectional phosphorelay network in Arabidopsis. *Current Biology*, *16*(11), 1116–1122.

Mao, D., Yu, F., Li, J., Van de Poel, B., Tan, D., Li, J., … Luan, S. (2015). FERONIA receptor kinase interacts with S-adenosylmethionine synthetase and suppresses S-adenosylmethionine production and ethylene biosynthesis in Arabidopsis. *Plant, Cell & Environment*, *38*(12), 2566–2574.

Martins, S., Dohmann, E. M., Cayrel, A., Johnson, A., Fischer, W., Pojer, F., … Vert, G. (2015). Internalization and vacuolar targeting of the brassinosteroid hormone receptor BRI1 are regulated by ubiquitination. *Nature Communications*, *6*, 6151.

Masachis, S., Segorbe, D., Turrà, D., Leon-Ruiz, M., Fürst, U., El Ghalid, M., … Di Pietro, A. (2016). A fungal pathogen secretes plant alkalinizing peptides to increase infection. *Nature Microbiology*, *1*, 16043.

Matos, J. L., Fiori, C. S., Silva-Filho, M. C., & Moura, D. S. (2008). A conserved dibasic site is essential for correct processing of the peptide hormone AtRALF1 in Arabidopsis thaliana. *FEBS Letters*, *582*(23–24), 3343–3347.

Matsubayashi, Y., Ogawa, M., Kihara, H., Niwa, M., & Sakagami, Y. (2006). Disruption and overexpression of Arabidopsis phytosulfokine receptor gene affects cellular longevity and potential for growth. *Plant Physiology*, *142*(1), 45–53.

Matsubayashi, Y., Ogawa, M., Morita, A., & Sakagami, Y. (2002). An LRR receptor kinase involved in perception of a peptide plant hormone, phytosulfokine. *Science*, *296*(5572), 1470–1472.

Matsubayashi, Y., & Sakagami, Y. (1996). Phytosulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of Asparagus officinalis L. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(15), 7623–7627.

Meng, X., Chen, X., Mang, H., Liu, C., Yu, X., Gao, X., … Shan, L. (2015). Differential function of Arabidopsis SERK family receptor-like kinases in stomatal patterning. *Current Biology*, *25*(18), 2361–2372.

Meng, X., Zhou, J., Tang, J., Li, B., de Oliveira, M. V., Chai, J., … Shan, L. (2016). Ligand-induced receptor-like kinase complex regulates floral organ abscission in Arabidopsis. *Cell Reports*, *14*(6), 1330–1338.

Miller, C., Skoog, F., Okumura, F., Von Saltza, M., & Strong, F. (1956). Isolation, structure and synthesis of kinetin, a substance promoting cell division. *Journal of the American Chemical Society*, *78*, 1375–1380.

Miyazaki, S., Murata, T., Sakurai-Ozato, N., Kubo, M., Demura, T., Fukuda, H., & Hasebe, M. (2009). ANXUR1 and 2, sister genes to FERONIA/SIRENE, are male factors for coordinated fertilization. *Current Biology*, *19*(15), 1327–1331.

Nam, K. H., & Li, J. (2002). BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell*, *110*(2), 203–212.

Nelson, D. C., Scaffidi, A., Dun, E. A., Waters, M. T., Flematti, G. R., Dixon, K. W., … Smith, S. M. (2011). F-box protein MAX2 has dual roles in karrikin and strigolactone signaling in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(21), 8897–8902.

Noguchi, T., Fujioka, S., Takatsuto, S., Sakurai, A., Yoshida, S., Li, J., & Chory, J. (1999). Arabidopsis det2 is defective in the conversion of (24R)-24-methylcholest-4-En-3-one to (24R)-24-methyl-5alpha-cholestan-3-one in brassinosteroid biosynthesis. *Plant Physiology*, *120*(3), 833–840.

Oh, M. H., Wang, X., Clouse, S. D., & Huber, S. C. (2012). Deactivation of the Arabidopsis BRASSINOSTEROID INSENSITIVE 1 (BRI1) receptor kinase by autophosphorylation within the glycine-rich loop. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(1), 327–332.

Ohyama, K., Ogawa, M., & Matsubayashi, Y. (2008). Identification of a biologically active, small, secreted peptide in Arabidopsis by in silico gene screening, followed by LC-MS-based structure analysis. *The Plant Journal*, *55*(1), 152–160.

Okuda, S., Tsutsui, H., Shiina, K., Sprunck, S., Takeuchi, H., Yui, R., … Higashiyama, T. (2009). Defensin-like polypeptide LUREs are pollen tube attractants secreted from synergid cells. *Nature*, *458*(7236), 357–361.

Olsen, J. V., Blagoev, B., Gnad, F., Macek, B., Kumar, C., Mortensen, P., & Mann, M. (2006). Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. *Cell*, *127*(3), 635–648.

Park, S. Y., Fung, P., Nishimura, N., Jensen, D. R., Fujii, H., Zhao, Y., … Cutler, S. R. (2009). Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science*, *324*(5930), 1068–1071.

Park, S. Y., Peterson, F. C., Mosquna, A., Yao, J., Volkman, B. F., & Cutler, S. R. (2015). Agrochemical control of plant water use using engineered abscisic acid receptors. *Nature*, *520*(7548), 545–548.

Pearce, G., Moura, D. S., Stratmann, J., & Ryan, C. A. (2001a). Production of multiple plant hormones from a single polyprotein precursor. *Nature*, *411*(6839), 817–820.

Pearce, G., Moura, D. S., Stratmann, J., & Ryan, C. A., Jr. (2001b). RALF, a 5-kDa ubiquitous polypeptide in plants, arrests root growth and development. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(22), 12843–12847.

Pearce, G., & Ryan, C. A. (2003). Systemic signaling in tomato plants for defense against herbivores. Isolation and characterization of three novel defense-signaling glycopeptide hormones coded in a single precursor gene. *The Journal of Biological Chemistry*, *278*(32), 30044–30050.

Pearce, G., Strydom, D., Johnson, S., & Ryan, C. A. (1991). A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *Science*, *253*(5022), 895–897.

Piotrowski, M., Liss, H., & Weiler, E. (1996). Touch-induced protein phosphorylation in mechanosensitive tendrils of Bryonia dioica Jacq. *Journal of Plant Physiology*, *147*, 539–546.

Qiao, H., Shen, Z., Huang, S. S., Schmitz, R. J., Urich, M. A., Briggs, S. P., & Ecker, J. R. (2012). Processing and subcellular trafficking of ER-tethered EIN2 control response to ethylene gas. *Science*, *338*(6105), 390–393.

Romanov, G. A., Lomin, S. N., & Schmulling, T. (2006). Biochemical characteristics and ligand-binding properties of Arabidopsis cytokinin receptor AHK3 compared to CRE1/AHK4 as revealed by a direct binding assay. *Journal of Experimental Botany*, *57*(15), 4051–4058.

Rotman, N., Gourgues, M., Guitton, A. E., Faure, J. E., & Berger, F. (2008). A dialogue between the SIRENE pathway in synergids and the fertilization independent seed pathway in the central cell controls male gamete release during double fertilization in Arabidopsis. *Molecular Plant*, *1*(4), 659–666.

Rotman, N., Rozier, F., Boavida, L., Dumas, C., Berger, F., & Faure, J. E. (2003). Female control of male gamete delivery during fertilization in Arabidopsis thaliana. *Current Biology*, *13*(5), 432–436.

Ruegger, M., Dewey, E., Gray, W. M., Hobbie, L., Turner, J., & Estelle, M. (1998). The TIR1 protein of Arabidopsis functions in auxin response and is related to human SKP2 and yeast grr1p. *Genes & Development*, *12*(2), 198–207.

Russinova, E., Borst, J. W., Kwaaitaal, M., Cano-Delgado, A., Yin, Y., Chory, J., & de Vries, S. C. (2004). Heterodimerization and endocytosis of Arabidopsis brassinosteroid receptors BRI1 and AtSERK3 (BAK1). *Plant Cell*, *16*(12), 3216–3229.

Santiago, J., Brandt, B., Wildhagen, M., Hohmann, U., Hothorn, L. A., Butenko, M. A., & Hothorn, M. (2016). Mechanistic insight into a peptide hormone signaling complex mediating floral organ abscission. *eLife*, *5*, e15075.

Schaller, G. E., & Bleecker, A. B. (1995). Ethylene-binding sites generated in yeast expressing the Arabidopsis ETR1 gene. *Science*, *270*(5243), 1809–1811.

Schallus, T., Feher, K., Sternberg, U., Rybin, V., & Muhle-Goll, C. (2010). Analysis of the specific interactions between the lectin domain of malectin and diglucosides. *Glycobiology*, *20*(8), 1010–1020.

Schallus, T., Jaeckh, C., Feher, K., Palma, A. S., Liu, Y., Simpson, J. C., … Muhle-Goll, C. (2008). Malectin: A novel carbohydrate-binding protein of the endoplasmic reticulum and a candidate player in the early steps of protein N-glycosylation. *Molecular Biology of the Cell*, *19*(8), 3404–3414.

Scheer, J. M., Pearce, G., & Ryan, C. A. (2005). LeRALF, a plant peptide that regulates root growth and development, specifically binds to 25 and 120 kDa cell surface membrane proteins of Lycopersicon peruvianum. *Planta*, *221*(5), 667–674.

Scheer, J. M., & Ryan, C. A. (1999). A 160-kD systemin receptor on the surface of lycopersicon peruvianum suspension-cultured cells. *Plant Cell*, *11*(8), 1525–1536.
Scheitz, K., Luthen, H., & Schenck, D. (2013). Rapid auxin-induced root growth inhibition requires the TIR and AFB auxin receptors. *Planta*, *238*(6), 1171–1176.
Schenck, D., Christian, M., Jones, A., & Luthen, H. (2010). Rapid auxin-induced cell expansion and gene expression: A four-decade-old question revisited. *Plant Physiology*, *152*(3), 1183–1185.
Schopfer, C. R., Nasrallah, M. E., & Nasrallah, J. B. (1999). The male determinant of self-incompatibility in Brassica. *Science*, *286*(5445), 1697–1700.
Schulze-Muth, P., Irmler, S., Schroder, G., & Schroder, J. (1996). Novel type of receptor-like protein kinase from a higher plant (Catharanthus roseus). cDNA, gene, intramolecular autophosphorylation, and identification of a threonine important for auto- and substrate phosphorylation. *The Journal of Biological Chemistry*, *271*(43), 26684–26689.
Shao, W., & Brown, M. (2004). Advances in estrogen receptor biology: Prospects for improvements in targeted breast cancer therapy. *Breast Cancer Research*, *6*(1), 39–52.
She, J., Han, Z., Kim, T. W., Wang, J., Cheng, W., Chang, J., … Chai, J. (2011). Structural insight into brassinosteroid perception by BRI1. *Nature*, *474*(7352), 472–476.
Sheard, L. B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T. R., … Zheng, N. (2010). Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. *Nature*, *468*(7322), 400–405.
Shih, H. W., Miller, N. D., Dai, C., Spalding, E. P., & Monshausen, G. B. (2014). The receptor-like kinase FERONIA is required for mechanical signal transduction in Arabidopsis seedlings. *Current Biology*, *24*(16), 1887–1892.
Shinohara, H., & Matsubayashi, Y. (2015). Reevaluation of the CLV3-receptor interaction in the shoot apical meristem: Dissection of the CLV3 signaling pathway from a direct ligand-binding point of view. *The Plant Journal*, *82*(2), 328–336.
Smalle, J., & Vierstra, R. D. (2004). The ubiquitin 26S proteasome proteolytic pathway. *Annual Review of Plant Biology*, *55*, 555–590.
Srivastava, R., Liu, J. X., Guo, H., Yin, Y., & Howell, S. H. (2009). Regulation and processing of a plant peptide hormone, AtRALF23, in Arabidopsis. *The Plant Journal*, *59*(6), 930–939.
Stirnberg, P., van De Sande, K., & Leyser, H. M. (2002). MAX1 and MAX2 control shoot lateral branching in Arabidopsis. *Development*, *129*(5), 1131–1141.
Sugano, S. S., Shimada, T., Imai, Y., Okawa, K., Tamai, A., Mori, M., & Hara-Nishimura, I. (2010). Stomagen positively regulates stomatal density in Arabidopsis. *Nature*, *463*(7278), 241–244.
Sun, Y., Han, Z., Tang, J., Hu, Z., Chai, C., Zhou, B., & Chai, J. (2013). Structure reveals that BAK1 as a co-receptor recognizes the BRI1-bound brassinolide. *Cell Research*, *23*(11), 1326–1329.
Symons, G. M., Ross, J. J., Jager, C. E., & Reid, J. B. (2008). Brassinosteroid transport. *Journal of Experimental Botany*, *59*(1), 17–24.
Tabata, R., Sumida, K., Yoshii, T., Ohyama, K., Shinohara, H., & Matsubayashi, Y. (2014). Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. *Science*, *346*(6207), 343–346.
Takayama, S., Shimosato, H., Shiba, H., Funato, M., Che, F. S., Watanabe, M., … Isogai, A. (2001). Direct ligand-receptor complex interaction controls Brassica self-incompatibility. *Nature*, *413*(6855), 534–538.
Takeuchi, H., & Higashiyama, T. (2016). Tip-localized receptors control pollen tube growth and LURE sensing in Arabidopsis. *Nature*, *531*(7593), 245–248.
Tang, W., Deng, Z., Oses-Prieto, J. A., Suzuki, N., Zhu, S., Zhang, X., … Wang, Z. Y. (2008). Proteomics studies of brassinosteroid signal transduction using prefractionation and two-dimensional DIGE. *Molecular & Cellular Proteomics*, *7*(4), 728–738.

Tang, J., Han, Z., Sun, Y., Zhang, H., Gong, X., & Chai, J. (2015). Structural basis for recognition of an endogenous peptide by the plant receptor kinase PEPR1. *Cell Research*, *25*(1), 110–120.

Thynne, E., Saur, I. M., Simbaqueba, J., Ogilvie, H. A., Gonzalez-Cendales, Y., Mead, O., … Solomon, P. S. (2016). Fungal phytopathogens encode functional homologues of plant rapid alkalinisation factor (RALF) peptides. *Molecular Plant Pathology*. http://dx.doi.org/10.1111/mpp.12444 [Epub ahead of print].

Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M., … Matsuoka, M. (2005). GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. *Nature*, *437*(7059), 693–698.

Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., … Yamaguchi, S. (2008). Inhibition of shoot branching by new terpenoid plant hormones. *Nature*, *455*(7210), 195–200.

Umezawa, T., Sugiyama, N., Takahashi, F., Anderson, J. C., Ishihama, Y., Peck, S. C., & Shinozaki, K. (2013). Genetics and phosphoproteomics reveal a protein phosphorylation network in the abscisic acid signaling pathway in Arabidopsis thaliana. *Science Signaling*, *6*(270), rs8.

Wang, X., Goshe, M. B., Soderblom, E. J., Phinney, B. S., Kuchar, J. A., Li, J., … Clouse, S. D. (2005). Identification and functional analysis of in vivo phosphorylation sites of the Arabidopsis BRASSINOSTEROID-INSENSITIVE1 receptor kinase. *Plant Cell*, *17*(6), 1685–1703.

Wang, X., Kota, U., He, K., Blackburn, K., Li, J., Goshe, M. B., … Clouse, S. D. (2008). Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. *Developmental Cell*, *15*(2), 220–235.

Wang, J., Lee, C., Replogle, A., Joshi, S., Korkin, D., Hussey, R., … Mitchum, M. G. (2010). Dual roles for the variable domain in protein trafficking and host-specific recognition of Heterodera glycines CLE effector proteins. *The New Phytologist*, *187*(4), 1003–1017.

Wang, J., Li, H., Han, Z., Zhang, H., Wang, T., Lin, G., … Chai, J. (2015). Allosteric receptor activation by the plant peptide hormone phytosulfokine. *Nature*, *525*(7568), 265–268.

Wang, T., Liang, L., Xue, Y., Jia, P. F., Chen, W., Zhang, M. X., … Yang, W. C. (2016). A receptor heteromer mediates the male perception of female attractants in plants. *Nature*, *531*(7593), 241–244.

Wang, Z. Y., Seto, H., Fujioka, S., Yoshida, S., & Chory, J. (2001). BRI1 is a critical component of a plasma-membrane receptor for plant steroids. *Nature*, *410*(6826), 380–383.

Wang, Y., Sun, S., Zhu, W., Jia, K., Yang, H., & Wang, X. (2013). Strigolactone/MAX2-induced degradation of brassinosteroid transcriptional effector BES1 regulates shoot branching. *Developmental Cell*, *27*(6), 681–688.

West, A. H., & Stock, A. M. (2001). Histidine kinases and response regulator proteins in two-component signaling systems. *Trends in Biochemical Sciences*, *26*(6), 369–376.

Wu, J., Kurten, E. L., Monshausen, G., Hummel, G. M., Gilroy, S., & Baldwin, I. T. (2007). NaRALF, a peptide signal essential for the regulation of root hair tip apoplastic pH in Nicotiana attenuata, is required for root hair development and plant growth in native soils. *The Plant Journal*, *52*(5), 877–890.

Wulfetange, K., Lomin, S. N., Romanov, G. A., Stolz, A., Heyl, A., & Schmulling, T. (2011). The cytokinin receptors of Arabidopsis are located mainly to the endoplasmic reticulum. *Plant Physiology*, *156*(4), 1808–1818.

Xie, D. X., Feys, B. F., James, S., Nieto-Rostro, M., & Turner, J. G. (1998). COI1: An Arabidopsis gene required for jasmonate-regulated defense and fertility. *Science*, *280*(5366), 1091–1094.

Xu, T., Dai, N., Chen, J., Nagawa, S., Cao, M., Li, H., … Yang, Z. (2014). Cell surface ABP1-TMK auxin-sensing complex activates ROP GTPase signaling. *Science*, *343*(6174), 1025–1028.

Yamaguchi, Y., Pearce, G., & Ryan, C. A. (2006). The cell surface leucine-rich repeat receptor for AtPep1, an endogenous peptide elicitor in Arabidopsis, is functional in transgenic tobacco cells. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(26), 10104–10109.

Yang, T., Wang, L., Li, C., Liu, Y., Zhu, S., Qi, Y., … Yu, F. (2015). Receptor protein kinase FERONIA controls leaf starch accumulation by interacting with glyceraldehyde-3-phosphate dehydrogenase. *Biochemical and Biophysical Research Communications*, *465*(1), 77–82.

Yu, F., Qian, L., Nibau, C., Duan, Q., Kita, D., Levasseur, K., … Luan, S. (2012). FERONIA receptor kinase pathway suppresses abscisic acid signaling in Arabidopsis by activating ABI2 phosphatase. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(36), 14693–14698.

Non-Print Items

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