Trends in Plant Science

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Review

Signaling Peptides and Receptors Coordinating Plant Root Development

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Small peptides mediate cell–cell communication to coordinate a variety of plant developmental processes. Signaling peptides specifically bind to the extracellular domains of receptors that belong to the receptor-like kinase family, and the peptide–receptor interaction activates a range of biochemical and physiological processes. The plant root is crucial for the anchorage of plants in soil as well as for the uptake of water and nutrients. Over recent years great progress has been made in the identification of receptors, structural analysis of peptide–receptor pairs, and characterization of their signaling pathways during plant root development. We review here recent advances in the elucidation of the functions and molecular mechanisms of signaling peptides, the peptide–receptor pairs that activate signal initiation, and their signaling pathways during root development.

Highlights

Small peptides are emerging as key signaling molecules in coordinating various aspects of plant root development.

Small signaling peptides are usually recognized by the extracellular domains of transmembrane proteins belonging to the receptor-like kinase family, and SERK family receptor-like kinases function as coreceptors for the peptide–receptor-like kinase pair activation via peptide-induced heterodimerization and transphosphorylation.

Small signaling peptides and their receptors modulate plant root development in response to changing levels of nitrogen in the soil as well as to adverse environmental conditions.

Small peptides can act as both long-distance signals and as local signals for root responses, acting via receptors located in different plant tissues.

Brief Introduction to Signaling Peptides

Small peptides are emerging as key signaling molecules that coordinate various aspects of developmental processes in plants. Systemin, that induces an immune response in young tomato plants, was the first identified peptide hormone in plants [1], and since then many other signaling peptides have been identified that play roles in a myriad of plant developmental processes [2–4]. Signaling peptides are categorized into secreted peptides and non-secreted peptides. Secreted signaling peptides can be further divided into two major classes: post-translationally modified peptides and cysteine-rich peptides (CRPs) [3]. Post-translationally modified signaling peptides are usually synthesized as larger inactive precursors harboring an N-terminal secretion sequence, a central variable region, and a C-terminal functional domain [3]. The precursor proteins undergo post-translational modifications such as tyrosine sulfation, proline hydroxylation, hydroxyproline arabinosylation, and proteolytic processing to produce functional small mature peptides of ~5 to 20 amino acids (aa) in length [3] (Figure 1). However, the order of the modifications and of processing and proteolysis is not clear in plants. By contrast, CRP peptides are characterized by the presence of 2–16 cysteine residues (typically 6 or 8) and intramolecular disulfide bonds, with or without proteolytic processing, and comprise 40 to >100 aa [3] (Figure 1).

Additional repertoires of peptide biosynthesis underlie the huge diversity of plant peptides. Some peptides are derived from functional proteins, and the biochemical activities of the derived peptides may differ from those of the original functional precursors. In addition, small open reading frames (sORFs) embedded in transcripts are additional sources of functional peptides. The sORFs are usually located in the 5′ leader sequence of the main ORFs in the primary transcript of miRNAs, as well as in other transcripts, and potentially encode peptides of 30–100 aa. They are directly translated without intermediate precursors and proteolytic processing, adding to the complexity of plant peptides [5]. More than 1000 signaling peptides

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have been predicted in the genome sequence of arabidopsis (*Arabidopsis thaliana*) [6], implying that peptide-derived intercellular communication is a prevailing signaling mechanism in plants.

Signaling peptides specifically bind to the extracellular domain of receptors, and the peptide–receptor interaction activates a range of biochemical and physiological processes [2]. To date, the identified receptors for peptide signals belong to the receptor-like kinase (RLK) family [7], particularly the **leucine-rich repeat receptor-like kinase** (LRR-RLK, see Glossary) subfamily comprising more than 200 members in arabidopsis [8]. Plant LRR-RLKs usually require shape-complementary **coreceptor** proteins for receptor activation. Signaling mediated by LRR-RLKs in many cases involves **BRASSINOSTEROID INSENSITIVE BRI1-ASSOCIATED RECEPTOR KINASE** (BAK1) and **SOMATIC EMBRYOGENESIS RECEPTOR KINASEs** (SERKs) as coreceptors [9–11]. Some LRR-receptor-like proteins (LRR-RLPs) (>50 members in arabidopsis) can also function as coreceptors [12].

We review here recent advances in the functional understanding of secreted signaling peptides and peptide–receptor pairs and how they initiate signaling activation, focusing in particular on plant root development.

Signaling Peptides in Primary Root Development

The Roles of RGF Peptides and Their Receptors in Root Meristem Maintenance and the Gravitropic Response

Tyrosine sulfation is a post-translational modification of peptide hormones that is mediated by tyrosylprotein sulfotransferase (TPST). The arabidopsis genome has only one *TPST* gene, and the loss-of-function mutant *tpst-1* shows pleiotropic phenotypes including severely shortened roots with a reduction in root **meristem** size [13]. Application of synthetic and sulfated ROOT MERISTEM GROWTH FACTOR 1 (RGF1) peptides restores root meristem activity in the *tpst-1* mutant, indicating that this peptide is required for **root stem cell** maintenance and that sulfation of the tyrosine residue of RGF1 is crucial for its activity [13]. In support, the *rgf1 rgf2 rgf3* triple mutant displays a short-root phenotype similar to that of the *tpst-1* mutant [13].

Three different groups independently identified receptors for RGFs using biochemical assays based on a signature ligand recognition motif, yeast two-hybrid screening with BAK1 as bait, and a combination of a **receptor kinase expression library** and **photoaffinity labeling**, respectively [14–16]. The *rgf insensitive* (*rgi*) quintuple mutants display a short primary root with a small-sized meristem [14,15]. The expression of *PLETHORA 1* (*PLT1*) and *PLT2* is almost undetectable in *rgi* quintuple mutants, and ectopic expression of *PLT2* in the quintuple mutant rescues the root meristem defects [14]. Genetic and expression analysis also showed that RGFR1, RGFR2, and RGFR3 are required for maintaining proper gradients of PLT transcription factors in the proximal meristem [15]. Thus, the RGF1 receptors play essential roles in RGF1–PLT-mediated root meristem development [14,15]. However, it remains to be clarified whether all five RGFRs are *bona fide* receptors that recognize the RGF1 peptide *in vivo*. Structural analysis and mutation studies revealed that the Arg-x-Gly-Gly motif is responsible for specific recognition of the sulfate group of RGF1 by RGFR1, and is important for RGFRs to distinguish RGFs from other similar peptides [16]. Gel filtration and coimmunoprecipitation analyses showed that RGF1 induces the interaction between RGFR1 or RGFR2 and SERK1/2/BAK1 [16]. Consistent with this, *serk* multiple mutants exhibit the phenotypes observed in the *rgfr* and *rgf1,2,3* mutants, such as smaller meristems and shorter roots than wild-type plants [16]. These *serk* mutants, except for *serk2 bak1*, have lost most of their responsiveness to RGF1 [16], indicating that SERKs are important for RGF1-induced signaling and act as coreceptors for RGFRs to regulate root meristem development. Together, these results have revealed a

Glossary

Auxin influx carrier: a class of auxin transporters that pump auxin from outside the cell to inside the cell.
BRASSINOSTEROID INSENSITIVE 1 (BRI1): the plasma membrane receptor protein for BR; belongs to the family of LRR receptor serine/threonine kinases. Upon BR binding, BRI1 heterodimerizes with the BAK1 coreceptor, forming an active complex for initiating BR signaling.
BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1): an LRR receptor serine/threonine kinase that associates with the brassinosteroid (BR) receptor, BRI1.
CLAVATA 3 (CLV3): a secreted peptide belonging to the family of CLE peptides that controls the size of the shoot apical meristem.
Columella cell: a cell located at the very tip of root; mediates the root gravitropic response.
Coreceptor: a receptor that interacts with a primary receptor to facilitate ligand perception and activate cellular signaling pathways.
Elongation/differentiation zone: a region in the root where cells elongate rapidly without growth in the transverse direction.
Leucine-rich repeat receptor-like kinase (LRR-RLK): a class of transmembrane proteins consisting of an extracellular domain containing leucine-rich repeats (LRRs), a membrane-spanning domain, and an intracellular serine/threonine kinase domain.
Meristem: a tissue made up of undifferentiated and dividing cells in plants; gives rise to various new tissues.
Organ abscission: a developmental process whereby plants actively shed unwanted organs.
Pericycle: a thin layer of tissue that gives rise to lateral roots (LRs), surrounded by the inner cortical layer, the endodermis, in the root, that is divided into two populations – one at the xylem pole and one at the phloem pole.
Photoaffinity labeling: upon treatment with UV light, the azide group of a peptide analog derivatized with ^{125}I-labeled photoreactive 4-azidosalicylic acid photolyzes to form a reactive nitrene, which forms a covalent bond with proximal amino

peptide signaling pathway in which RGF peptides regulate primary root meristem activity through PLT transcription factors by interacting with RGFRs/BAK1 receptor pairs (Figure 2A).

Deregulation of the RGF/GOLVEN (GLV)/**CLAVATA 3** (CLV3)/EMBRYO SURROUNDING REGION (ESR)-RELATED (CLE)-LIKE (CLEL) function by gain-of-function and loss-of function mutations impaired the formation of auxin gradients and the gravitropic response, indicating that some GLV peptides, such as GLV1/RGF6/CLEL6, GLV2/RGF9/CLEL9, and GLV3/RGF4, are also involved in the gravitropic response by controlling the distribution of auxin [17]. Peptide treatments and mutant analysis suggested that RGF/GLVs control the abundance and trafficking dynamics of the auxin efflux carrier PINFORMED 2 in the root meristem by a post-transcriptional mechanism [17]. However, because no defects in gravitropic response have been noted in *rgfr* quintuple mutants [14,15,16], further investigation will be necessary to confirm the role of RGF1 and RGF1 receptor pairs in the gravitropic response. Two related subtilase genes, *AtSBT6.1* and *AtSBT6.2*, have been identified to produce the mature bioactive GLV1 peptide [18].

The Roles of CLE Peptides and Their Receptors in Root Meristem Maintenance and Protophloem Formation

Root meristem maintenance is also regulated by CLE peptides. These CLE peptides have a conserved 12–14 aa CLE motif near the C-terminus [19]. Thirty-two CLE peptide genes have been identified in arabidopsis, and their peptides can be divided into two groups: type A CLEs and type B CLEs [20]. Type A CLE peptides regulate root meristem development, whereas type B CLE peptides are involved in vascular development. CLE40, a type A CLE peptide and the closest homolog of CLV3, is required for differentiation of **columella cells**, because the *cle40* loss-of-function mutant exhibits a short-root phenotype with irregularly shaped root tips owing to the delayed differentiation of columella cells [21]. Both *clv1* and *arabidopsis crinkly 4* (*acr4*) single mutants have additional columella stem cell layers, and *acr4* is insensitive to the CLE40 peptide [21,22], indicating that CLV1 and ACR4 receptor kinases mediate CLE40 signaling. CLV1 and ACR4 form homo- and heterodimeric complexes [22]. A synthetic CLE-domain peptide of CLE40 can bind to CLV1 *in vitro* [23]. Thus the heterodimeric CLV1–ACR4 receptor complex may recognize CLE40 peptide. Homeodomain protein WUSCHEL RELATED HOMEOBOX (WOX) 5 acts in the **quiescent center** (QC) to maintain adjacent columella stem cell activity [24]. *WOX5* expression is altered in the *cle40* mutant, and extends beyond the QC, but is reduced in the QC when CLE40 levels are increased [21], indicating that CLE40 suppresses *WOX5* expression to control distal stem cell homeostasis. These results suggest that the CLE40–ACR4–WOX5 signaling pathway is important for maintaining the stem cell niche and for controlling columella cell development (Figure 2A).

Some CLEs control primary root growth through the regulation of protophloem formation during vascular development in arabidopsis [25–28]. The roots of arabidopsis LRR-RLK gene *barley any meristem 3* (*BAM3*) mutants specifically resist protophloem differentiation and growth inhibition by CLE45 peptide, indicating that suppression of protophloem differentiation and root meristem growth by CLE45 requires BAM3 [25]. Genetic analysis indicated that quantitative interplay between two opposing signaling pathways of *BAM3*-mediated CLE45 and *OCTUPUS* (*OPS*) determines cellular commitment to protophloem sieve element fate [26]. BAM3 has been shown to be a *bona fide* CLE45 receptor, acting independently of SERKs [28]. However, CLV2 and the pseudokinase CORYNE (CRN) are required to fully sense root-active CLE peptides, including CLE45, possibly by stabilizing the expression of their receptors, although CLV2 and CRN are not involved in the direct perception of CLE peptides [28]. Tightly balanced levels of phosphatidylinositol-4,5-biphosphate genetically acting upstream of *OPS*

acid residues within the binding sites of receptor proteins, and thus labels the receptor proteins.

Quiescent center (QC): a group of cells at the root tip which rarely divide; these maintain the adjacent stem cell population.

Receptor kinase expression library: overexpression lines of receptor kinases in tobacco BY-2 cells.

Root stem cell: innately undifferentiated cells located in the root meristems of plants, which maintain themselves while providing a steady supply of precursor cells to form differentiated root tissues and organs.

SOMATIC EMBRYOGENESIS RECEPTOR KINASEs (SERKs): a family of five LRR-receptor kinases involved in at least five different plant signaling pathways. One member of this family is BAK1, also known as SERK3.

Subtilisin-like proteinase: a type of serine protease that is ubiquitous in both prokaryotes and eukaryotes.

Systemic nitrogen (N)-acquisition response: a response whereby N starvation on one side of the root promotes the uptake of N on the other side of the root in N-rich conditions.

Tracheary elements: nonliving, water-conducting cells with a secondary cell wall in the xylem.

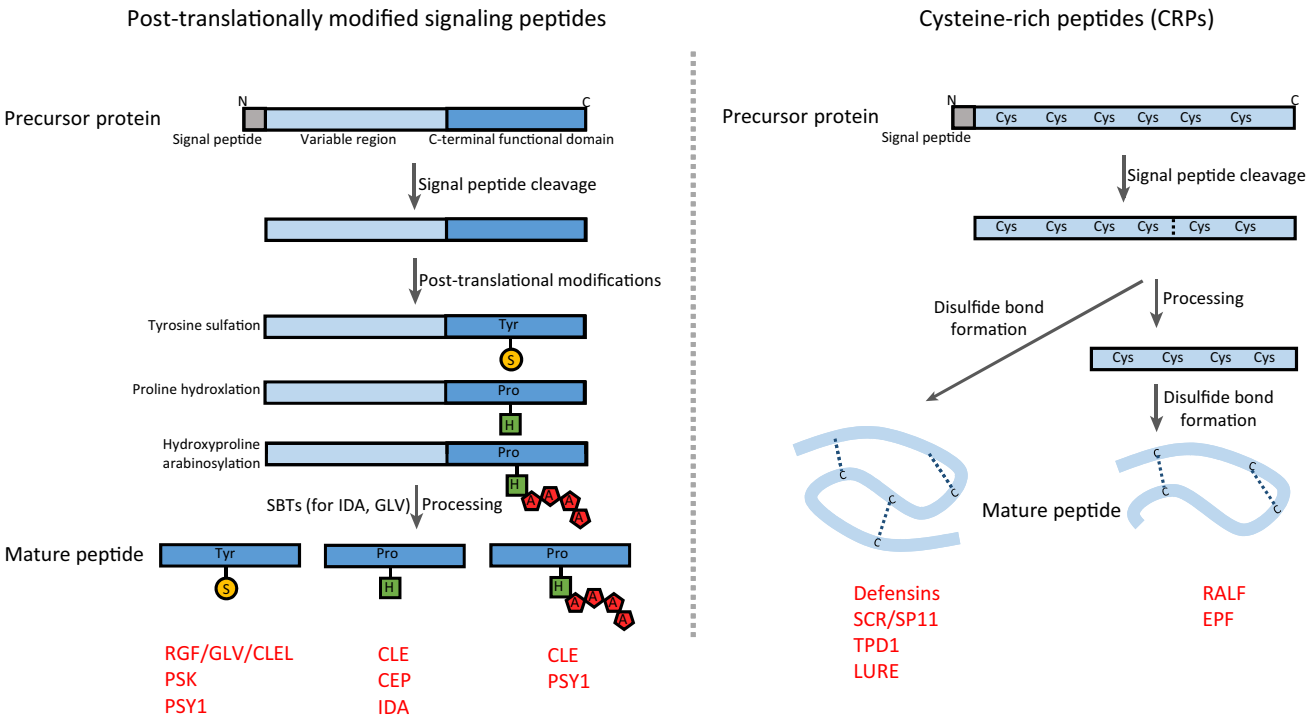

Figure 1. Structural Categorization and Maturation of Small Signaling Peptides. Precursor proteins of signaling peptides undergo post-translational modifications, such as tyrosine sulfation, proline hydroxylation, and hydroxyproline arabinosylation after cleavage of the N-terminal signal peptide, followed by proteolytic processing to give rise to smaller mature peptides [3]. SBTs are involved in proteolytic processing of the GLV1 and IDA peptides [18,65]. The precursor proteins of CRPs undergo intramolecular disulfide bond formation with or without proteolytic processing. Abbreviations: A, arabinosylation; Cys, cysteine; CRP, Cys-rich peptide; H, hydroxylation; IDA, INFLORESCENCE DEFICIENT IN ABSCISSION; Pro, proline; SBT, subtilisin-like proteinase; S, sulfation; Tyr, tyrosine.

are essential for proper protophloem differentiation [27]. Interestingly, a higher density and accelerated emergence of LRs are produced by mutations in *COTYLEDON VASCULAR PATTERN 2* (*CVP2*) encoding a putative phosphoinositide 5-phosphatase, its homolog *CVP2-like 1* (*CLV1*), and *OPS*, and are also created by the application of CLE26 and CLE45, indicating that local changes in the primary root protophloem affect overall root architecture [27].

Among the various synthetic CLE peptides tested for the arabidopsis root response, CLE26 is the most effective in inducing the short-root phenotype [29]. Arabidopsis CLE26 also affects the root architecture of monocot plants such as *Brachypodium distachyon* and wheat, as well as of other agriculturally important species such as *Brassica napus* and *Solanum lycopersicum*, indicating that the role of CLE26 in regulating root architecture is conserved in monocot and other crop species [29,30]. *CLE26* expression is increased by auxin treatment and, conversely, CLE26 decreases the abundance of auxin transporter PIN1 protein without affecting *PIN1* transcript levels, indicating that auxin signaling is connected to the CLE26 pathway at the protein level [30].

The Roles of CEP Peptides and Their Receptors in the Systemic Nitrogen (N)-Acquisition Response

Ohyama *et al*. (2008) used an *in silico* gene screening method in combination with liquid chromatography/mass spectrometry analysis to identify a novel gene family encoding small

secreted peptides with significant sequence similarities within the short conserved C-terminal domain [31]. C-TERMINALLY ENCODED PEPTIDE 1 (CEP1), a peptide encoded by one of these genes, is a 15 aa peptide containing two hydroxyproline residues. *CEP1* overexpression or application of CEP1 peptides significantly suppressed primary root growth by repressing cell division in the meristem zone without affecting the QC region, indicating that CEP1 is involved in primary root growth and development by regulating root meristem activity [31]. CEPs are also involved in the **systemic N-acquisition response**. N, a vital nutrient for plants, is often distributed unevenly in the soil, and thus plants have evolved a systemic mechanism by which N starvation on one side of the root system leads to increased nitrate uptake on the other side [32]. At least seven of the 15 *CEP* genes in arabidopsis are upregulated specifically in response to N starvation in roots. Rootlets starved of N secrete CEPs that are translocated to the shoot and recognized by two LRR-RKs, CEP RECEPTOR 1 and 2 (CEPR1 and CEPR2) (Figure 2A).

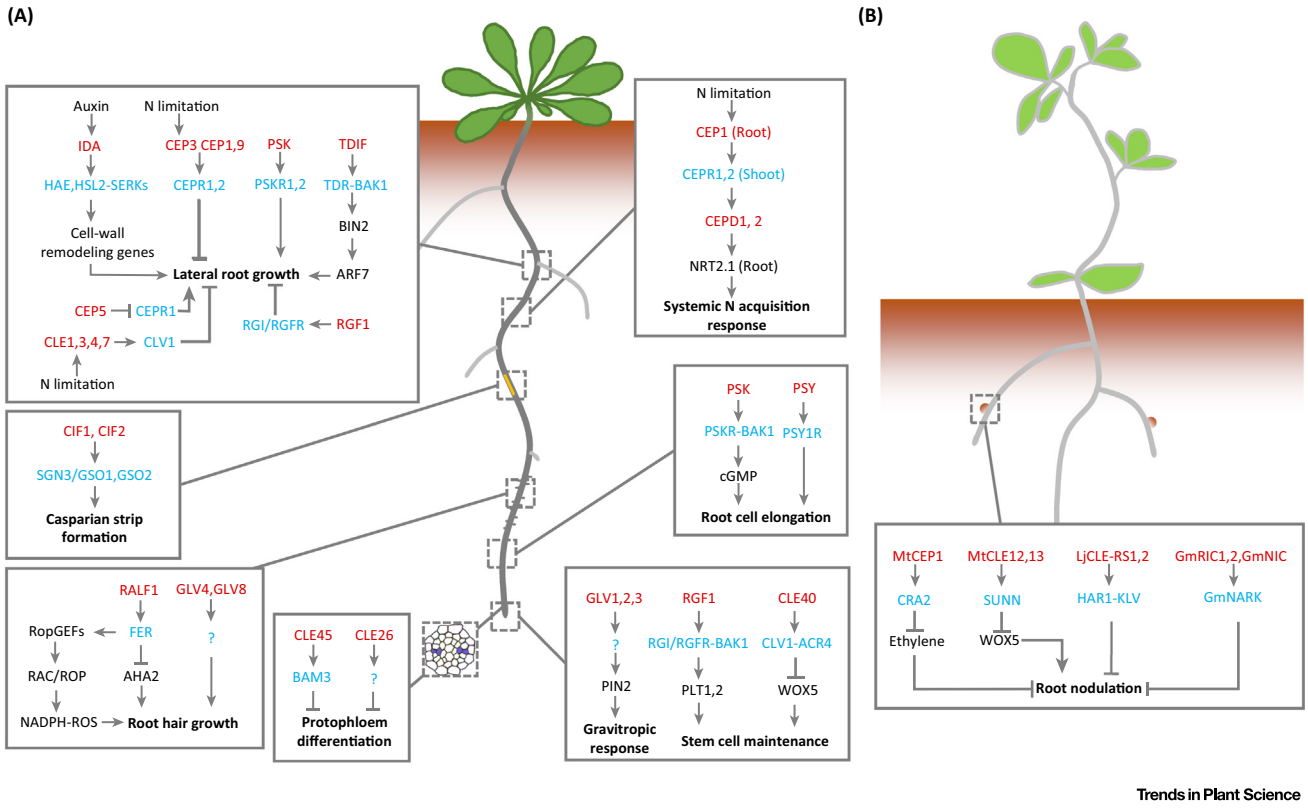

Figure 2. Multiple Functions of Signaling Peptides in Root Development

For a Figure360 author presentation of Figure 2, see the figure legend at https://doi.org/10.1016/j.tplants.2017.12.007

(A) Signaling peptides and their receptors involved in lateral root growth, Casparian strip formation, root hair formation, the systemic nitrogen acquisition response, root cell elongation in the elongation/differentiation zone, the root gravitropic response, and root stem cell maintenance in arabidopsis. Peptides and their receptors are depicted in red and blue, respectively. Each panel shows cellular signaling pathways of the small peptide and receptor pairs coordinating various aspects of root development in arabidopsis. Abbreviations: AHA2, H^+-ATPase 2; cGMP, cyclic guanosine monophosphate; GEFs, guanine nucleotide exchange factors; N, nitrogen; NADPH-ROS, NADPH-dependent reactive oxygen species; RAC/ROP, RAC/ROP GTPases. Abbreviations of peptide and receptor names are given in the text. (B) Signaling peptides and receptors involved in root nodulation in leguminous plants. The homologs of CLE peptides and CLV receptors in leguminous plants regulate root nodulation. Peptides and receptors: GmRIC1, GmRIC2, and GmNIC, CLE peptides in *Glycine max*; GmNARK, CLV1 homolog in *Glycine max*; LjCLE-RS1 and LjCLE-RS2, CLE peptides in *Lotus japonicus*; HAR1, CLV1 homolog in *Lotus japonicus*; KLV, RPK2 homolog in *Lotus japonicus*; MtCLE12 and MtCLE13, CLE peptides in *Medicago truncatula*; SUNN, CLV1 homolog in *Medicago truncatula*; MtCEP1, CEP peptide in *Medicago truncatula*; CRA2, CEPR1 homolog in *Medicago truncatula*.

The CEP–CEPR pair in the shoot generates mobile N-demand signals to systemically upregulate the nitrate transporter genes in the roots [32]. Recently, it was revealed that the phloem-specific polypeptides, CEP DOWNSTREAM 1 and 2 (CEPD1 and CEPD2), are the descending shoot-to-root mobile signals and are induced by the perception of CEP1 by CEPR1 [33]. The resulting CEPD peptides are then translocated to the roots and upregulate expression of the nitrate transporter gene *NRT2.1* [33] (Figure 2A).

The Roles of PSK and PSY1 Peptides and Their Receptors in Cell Expansion during Root Growth

The tyrosine-sulfated peptides, phytosulfokine (PSK) and PLANT PEPTIDE CONTAINING SULFATED TYROSINE 1 (PSY1), can promote primary root growth by regulating cell expansion in the **elongation/differentiation zone** (Figure 2A). PSK is a secreted 5 aa sulfated peptide that was initially identified as a growth-promoting peptide in plant cell cultures [3]. PSY1 is an 18 aa secreted sulfated glycopeptide that significantly promotes cellular proliferation and expansion at nanomolar concentrations [34]. Two LRR-RKs, PSKR1 and PSKR2, recognize PSK, and their homolog PSY1R is required for PSY1 perception [34]. Analysis of *pskr1 pskr2* double mutants and *pskr1 pskr2 psyr1* triple mutants indicated that these LRR-RKs integrate growth-promoting signals mediated by two structurally distinct peptides, PSK and PSY1 [34]. The PSKRs have kinase, guanylate cyclase, and Ca^{2+}/CaM-binding activities that are essential for the PSK response [35,36]. PSKR1-deficient protoplasts do not expand in response to PSK but are still responsive to cGMP [37], suggesting that cGMP acts downstream of PSKR1. The roots of de-etiolated *bak1* mutant seedlings are unresponsive to PSK, and *bak1* protoplasts expand less in response to PSK but are fully responsive to cGMP [37], indicating that BAK1 acts in the PSK signaling pathway upstream of cGMP. Yeast two-hybrid and colocalization assays indicated that PSKR1 interacts with BAK1/SERK3 [37]. Structural, biochemical, and genetic evidence suggests that PSK binding to PSKR stabilizes the PSKR island domain and enhances PSKR heterodimerization with the SERKs [10].

The Roles of CIF1/CIF2 Peptides and Their Receptors in Casparian Strip Formation

The Casparian strip functions as a hydrophobic barrier embedded within the walls of endodermal cells to prevent passive apoplastic diffusion of ions and water across the endodermis in the root. Two endodermis-expressed LRR-RKs, SCHENGEN 3/GASSHO 1 (SGN3/GSO1) and GSO2, are required for contiguous Casparian strip formation [38]. Recently, peptides that directly bind to these LRR-RKs, CASPARIAN STRIP INTEGRITY FACTOR 1 (CIF1) and CIF2, were identified [39,40]. Mutants devoid of CIF peptides are defective in Casparian strip formation and exhibit severely stunted growth at high concentrations of iron, but these defects are fully restored by the application of CIF1 peptide [40]. The expression of *CIF1* and *CIF2* is upregulated by excess iron and is further synergistically regulated by lowering the pH of the medium, making iron more soluble, indicating that this response may reflect an adaptation to ensure the growth and survival of the plants under unfavorable mineral conditions [40]. SGN1, a receptor-like cytoplasmic kinase, is required to transduce CIF1-activated SGN3 signals [39,41]. However, SGN1 is observed exclusively at the outer, cortex-facing side of the endodermal plasma membrane, whereas CIF1 peptides are produced in inner, stellar cell layers [39,41]. Thus the CIF–SGN3–SGN1 signaling module operates only when the Casparian strip is not established. Once sealed, CIFs will no longer be able to reach the domain where SGN3 and SGN1 can colocalize, leading to strongly reduced signaling. However, this signaling module will be reactivated whenever the endodermal diffusion barrier becomes compromised. Therefore, the CIF–GSO pair acts as a barrier surveillance system to guarantee sealing of the Casparian strip network in response to root developmental cues and adverse soil conditions [39–41] (Figure 2A).

Signaling Peptides in Lateral Root (LR) Development

The Roles of CLE and CEP Peptides and Their Receptors in N-Responsive LR Growth

Some CLE peptides function in LR growth in response to changing levels of N in soil. *CLE1*, *CLE3*, *CLE4*, and *CLE7* are expressed predominantly in the **pericycle**, and their expression in arabidopsis roots is downregulated by N supply, and is upregulated by low levels of N [42]. Overexpression of these *CLE* genes inhibits LR growth [42], indicating that these CLE peptides act as negative regulators of LR growth. Because the *clv1-1* mutant has longer LRs under low-N conditions, CLV1 may be a receptor for CLE peptides [42]. Root-specific expression of *CLE3* inhibits LR formation in the wild type but not in the *clv1* mutant, indicating that CLE-dependent inhibition of LR formation requires CLV1 [42]. These results show that a CLE–CLV1 signaling module is important in the regulation of LR growth in response to changing levels of N in the soil (Figure 2A).

CEP1 is expressed in the LR primordia, and overexpression of *CEP1* inhibits LR growth in arabidopsis [31]. Application of synthetic CEP peptides, such as CEP1, CEP3, and CEP9, disrupts LR development [31,43]. *CEP5* has also been shown to function as a negative regulator of LR formation [44]. In the legume *Medicago trancatula*, MtCEP1 also acts as a negative regulator of LR formation [45]. *CEP* genes are responsive to various abiotic stress conditions to varying degrees in roots [43]. In particular, *CEP3* expression is highly upregulated by N limitation in roots. The *cep3* mutant shows increased LR growth under a range of abiotic stress conditions, such as N limitation and salt and osmotic stress, and thus CEP3 may mediate environmental regulation of LR growth [43]. Two CEPR receptor kinases, CEPR1/XYLEM INTERMIXED WITH PHLOEM 1 (XIP1) and CEPR2, bind to several members of the CEP family [32,44]. Consistent with a role for *CEPs* in inhibiting LR growth, *cepr1* and *cepr1 cepr2* mutants exhibit enhanced LR growth [32], indicating that the CEP1–CEPR1/XIP1 module is important for controlling LR growth (Figure 2A). However, the CEP5–CEPR1/XIP1 interaction occurring at phloem pole pericycle cells seems to be an antagonistic peptide–receptor pair interaction in LR initiation (Figure 2A) because *CEP5* overexpression reduces LR density in a similar manner to the *cepr1/xip1* mutant, which displays a reduction in stages I and II LR primordia [44]. Therefore, two different peptides, CEPs and CLEs, inhibit LR growth and development in response to N limitation by interacting with their own distinct receptors.

CEPs produced under local N limitation act as long-distance signals through shoot-located CEPR1/XIP1 to generate CEPD1 and CEPD2, the descending mobile signals, to upregulate high-affinity nitrate transporter genes in roots [32,33]. CEP5 acts locally to inhibit LR initiation, which is dependent on root-located CEPR1/XIP1 [44]. Thus CEPs can have both long-distance and local effects on root response acting via receptors located in different plant tissues.

The Roles of TDIF Peptide and Its Receptor in LR Formation

A recent study showed that the TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR (TDIF)–TDIF RECEPTOR/PHLOEM INTERCALATED WITH XYLEM (TDR/PXY)–BRASSINOSTEROID INSENSITIVE 2 (BIN2)–AUXIN RESPONSE FACTOR (ARF) signaling pathway plays a role in LR formation [46] (Figure 2A and Box 1). TDIF is a 12 aa peptide containing two hydroxyproline residues, and is encoded by *CLE41* and *CLE44* [47]. TDIF is secreted from the phloem and perceived by TDR in xylem pole pericycle cells [46]. In the presence of TDIF, TDR interacts with BIN2 in the pericycle and enhances BIN2-mediated phosphorylation of ARFs to activate the ARFs [46]. Phosphorylation of ARFs interferes with interactions between ARFs and AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA), negative regulators of ARFs, allowing the formation of active ARFs that promote LR formation [46]. Treatment with TDIF increases LR density in a dose-dependent manner, and *tdr* mutant is insensitive to

Box 1. Lateral Root Development

Plant roots are mainly divided into primary roots and LRs. The primary root is formed from the radicle of the seed and grows vertically downwards into the soil, and is essential for anchorage to the soil and for absorbing water and nutrients. LRs are a major determinant of the root architecture in plants and are thus crucial for the uptake of water and nutrients [93]. In *Arabidopsis thaliana* LRs originate from founder cells formed from xylem pole pericycle cells primed in the basal meristem, and these cells undergo anticlinal and asymmetric division to create a single-layered primordium for LR initiation [94]. A series of coordinated periclinal and anticlinal divisions of this primordium give rise to a highly organized dome-shaped LR primordium that emerges from the primary root via cell separation. The plant hormone auxin plays crucial roles in nearly every step of LR formation [95]. Many components involved in LR development and signaling pathways have been identified in arabidopsis, including AUXIN/INDOLE-3-ACETIC ACID–AUXIN RESPONSE FACTOR modules via auxin transporters [95].

the TDIF treatment and exhibits altered LR density, showing that the TDIF–TDR pair functions in LR formation [46]. TDIF also activates the glycogen synthase kinase pathway, promoting cellular differentiation into **tracheary elements** [48]. The crystal structures of the TDR–TDIF complex revealed the recognition mechanism of TDIF by TDR [49,50]. A comparison of structures of plant LRR-RKs and their ligand complexes revealed conserved structural features among the plant LRR-RK family members, such as twisted superhelical structures and the N-terminal capping domain [7,49,50]. However, TDR has notable structural differences from other plant LRR-RKs, such as lack of an insertion domain for ligand-dependent heterodimerization with BAK1, and shorter LRR domains, such that TDR–TDIF forms a heterodimer with SERK1 but not with BAK1. By contrast, BRI1 forms a ligand-dependent heterodimer with the co-receptor BAK1 in which the ligand brassinolide is sandwiched between BRI1 and BAK1, serving as molecular glue [51].

The Roles of GLV/RGF/CLEL Peptides in LR Formation

Of 11 GLV/RGF/CLEL family members, 10 *GLV* genes are expressed during LR development in arabidopsis [18,52]. Overexpression of most *GLV* genes results in various root defects, including reduction in the number of emerging LRs and defects in pericycle cell division, while no detectable changes in LR formation occur in single loss-of-function mutations in each *GLV* gene [52–54]. Application of synthetic GLV peptides produces similar phenotypic changes in the wild type to those produced by *GLV* overexpression [53,54]. Thus, many of the GLV peptides likely play roles in LR development in arabidopsis. Analysis of multiple *glv* mutants will provide clues to the biological roles of GLV peptides in LR formation. RGF1 treatment greatly reduces LR numbers in arabidopsis, and the *rgi1,2,3,4* quadruple mutant exhibits reduced sensitivity to RGF1 in LR formation compared to the wild type, indicating that the RGF1 and RGF1 receptor pair plays a role in LR formation [14] (Figure 2A).

The Roles of RALFs during LR Initiation and Emergence

RAPID ALKALINIZATION FACTORs (RALFs) belong to the group of CRPs with proteolytic processing [55]. RALF peptides affect cell growth by regulating Ca^{2+} responses, MAPK signaling, and alkalization, and are linked to LR development [55–57]. Overexpression of *RALF1* reduces LR density, whereas knockdown of *RALF1* expression causes an increase in LR emergence, indicating that RALF1 has a negative regulatory role in LR emergence [57]. RALF1 and other RALFs, such as RALF19 and RALF23, have also been shown to be involved in LR initiation [56,57]. A RALF-LIKE peptide, RALFL34, is involved in regulating LR initiation. *RALFL34* is expressed in xylem pole pericycle cells, and *ralfl34* mutants exhibit abnormal pericycle division patterns and increased LR density [58], indicating that RALFL34 peptide plays a role in correct cell division in the pericycle during LR initiation.

The Roles of IDA Peptide and Its Receptor in LR Emergence

LRs pass through endodermal, cortical, and epidermal cell layers to emerge from the primary root, and therefore cell separation in these tissue layers overlaying the LR primordium is crucial for LR growth and emergence [59]. This emergence process resembles **organ abscission**, and the INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) peptide, which is involved in floral abscission [60], regulates LR emergence [61]. The **auxin influx carrier**-like AUX1-3-derived auxin in the neighboring cortical and epidermal cells triggers degradation of SOLITARY ROOT 1/IAA14, allowing ARFs to promote *IDA* expression [61]. IDA is perceived by the HAESA (HAE) and HAESA-LIKE 2 (HSL2) LRR-RLKs [62], and facilitates cell-wall degradation by inducing the expression of various cell-wall remodeling enzymes, promoting LR emergence [61] (Figure 2A). SERK1 acts as a HAESA coreceptor and promotes high-affinity IDA-sensing during floral organ abscission [63]. Genetic analyses revealed that all four SERK family RLKs function redundantly in regulating floral organ abscission upstream of the MAP kinase cascade [64]. IDA induces heterodimerization of HAE/HSL2 and SERKs, which transphosphorylate each other [64]. However, it is not known whether SERKs function with HAE/HSL2 to promote LR emergence. **Subtilisin-like proteinases** form the mature and bioactive IDA from the precursor protein during floral abscission [65] (Figure 1), but it remains to be determined whether the same proteinases act to process the IDA precursor protein in LR formation.

The Roles of PSK Peptide in LR Growth and Formation

Arabidopsis has five *PSK* genes that are expressed in the LR primordia [66]. PSK peptides bind to PSKR1 and PSKR2 [67,68], and these PSK–PSKR pairs are important for primary root growth [66]. However, exogenous PSK treatment increases LR emergence and total LR length with increased cell size independently of PSKR1. Thus PSK may interact with other receptors to regulate LR development [66].

Signaling Peptides in Root Hair Formation

The Roles of RALF Peptide and Its Receptor in Root Hair Development

RALF peptides regulate root hair development (Box 2). Arabidopsis plants overexpressing *RALF1* show inhibited root hair growth [69], and application of synthetic RALF peptides produces a similar effect [70]. RALF peptides stimulate the phosphorylation of H^+-ATPase 2 (AHA2) to inhibit its proton pump activity, and the consequent increase in apoplastic pH reduces cell elongation [71], indicating that RALF peptides control root hair formation via the regulation of cell elongation. FERONIA (FER) is a receptor for RALF peptides [72]. The *fer* knockout mutants exhibit root hair defects and are insensitive to RALF treatment [72,73]. Activation of FER by RALF results in the phosphorylation of AHA2, which increases extracellular alkalinization, reducing cell elongation [71,74] (Figure 2A). FER also interacts with several plant-specific guanine nucleotide exchange factors (RopGEFs), such as RopGEF4 and RopGEF10, to activate the plant RHO GTPases (RAC/ROP) signaling pathway [72,73,75,76] (Figure 2A). Regulated RAC/ROP activity controls the production of NADPH-dependent reactive oxygen species (ROS) [72], and spatially regulated accumulation of ROS leads to enhancement of polarized root hair growth [73] (Figure 2A). The RALF–FER pair requires additional components, such as LORELEI-LIKE-GPI-ANCHORED PROTEIN 1 (LLG1) and RPM1-induced protein

Box 2. Root Hair Formation

Root hairs are cylindrical outgrowths from root epidermal cells that enable water uptake, nutrient acquisition, and environmental sensing in plants [96]. Root epidermal cells are arranged with alternating cell identities that produce root hairs or not [96]. Intercellular communication is important for specifying trichoblast cells located adjacent to cortical cells for root hair formation [97].

kinase (RIPK), to trigger activation of the GEF–ROP pathway that mediates auxin-dependent root hair development [74,77].

The Roles of GLV/RGF/CLEL Peptides in Root Hair Elongation

Some members of GLV/RGF/CLEL family, which is closely related to the CLE family, participate in root hair formation [52]. *GLV4* and *GLV8* are expressed in root cortical and non-hair cells. Ectopic expression of *GLV4* and *GLV8* produces abnormal root hairs with irregular shapes, whereas loss-of-function mutants display shorter root hairs without cell-fate changes [52]. Thus these peptides act to assist proper root hair elongation in a non-cell-autonomous manner (Figure 2A).

Signaling Peptides in Root Nodulation

The Roles of CLE Homologs and Their Receptors in Regulating Root Nodulation

Homologs of CLE peptides and CLV receptors [CLV1, Receptor-like protein kinase 2 (RPK2), and CLV2–CRN] regulate nodule numbers in legumes. The involvement of CLE signaling in the autoregulation of nodulation (AON) was first identified in soybean *Glycine max* (Box 3). Overexpression of soybean *RHIZOBIA-INDUCED CLE1*, *2*, and *Nitrate-Induced CLE* (*GmRIC1*, *GmRIC2*, and *GmNIC*) inhibits nodulation, whereas mutations in the soybean *CLV1*-homolog *NODULE AUTOREGULATION RECEPTOR KINASE* (*GmNARK*) result in hypernodulation [78]. Nodulation in *nark* mutants is not inhibited by GmCLE, indicating that the GmCLE–GmNARK pair regulates AON [78,79] (Figure 2B).

The CLE-dependent regulation of AON is observed in other legumes such as *Lotus japonicus* and *M. truncatula*. The *L. japonicus* genome contains at least 39 *LjCLE* genes [80]. Of these, the CLE peptides, LjCLE-Root Signal 1 (LjCLE-RS1) and LjCLE-RS2, are upregulated upon rhizobial infection through a NODULE INCEPTION (NIN)-dependent pathway [80,81]. Transformation of *L. japonicus* hairy roots with *LjCLE-RS1* and *LjCLE-RS2* locally and systemically reduces the number of nodules [80]. Application of a synthetic arabinosylated CLE-RS2 peptide suppresses nodulation [82]. LjCLE-RS1 and LjCLE-RS2, derived from the root, are transported through the xylem and are perceived in the shoot by the CLV1-homolog HYPERNODULATION ABERRANT ROOT FORMATION 1 (HAR1) LRR-RLK [80,82] (Figure 2B). KLAVIER (KLV), a homolog of arabidopsis RPK2, interacts with HAR1, and the *har1 klv* double mutant shows no additive nodulation phenotypes [83], indicating that they act in the same signaling pathway for AON (Figure 2B). MtCLE12 and MtCLE13 peptides, which are homologous to LjCLE-RS1 and LjCLE-RS2 and are expressed in root nodules, inhibit nodulation in *M. truncatula* [84] (Figure 2B). The systemic effects of MtCLE12 and MtCLE13 on nodulation are mediated by the shoot-expressed LRR-RLK SUPER NUMERIC NODULES (SUNN), a CLV1 homolog [84]. It has been shown that the CLE–CLV–WOX5 pathway controls AON in both *Medicago* and pea [85] (Figure 2B).

Box 3. Root Nodulation

Leguminous plants can establish a symbiosis with N-fixing soil bacteria to form symbiotic nodules on roots, which enables the host plants to grow in N-poor soil. Because excessive nodule formation is deleterious to plants in view of the high metabolic cost, the number of nodules is intricately controlled by external and internal factors [98]. While soil nitrate levels are a major environmental factor that regulates nodule formation, autoregulation of nodulation further refines nodulation via long-distance signaling between roots and shoots [98]. A root-derived signal generated by root nodulation is perceived by the shoots, and a subsequent shoot-derived signal limits the number of nodules in the root [98].

The Roles of CEP1 Homolog and Its Receptor in Regulating Root Nodulation

MtCEP1 acts as a positive regulator of nodule formation independently of AON in *Medicago*. In *Medicago*, overexpression of *MtCEP1* promotes nodule formation [45], whereas a loss-of-function mutant of *COMPACT ROOT ARCHITECTURE 2* (*CRA2*), a *CEPR1* homolog, displays a reduced number of nodules and is unresponsive to MtCEP1 [86]. These observations indicate that MtCEP1 is a positive regulator of nodulation and that CRA2 may perceive MtCEP1 (Figure 2B). The MtCEP1–MtCRA2 pair suppresses ETHYLENE INSENSITIVE 2/SKL-mediated ethylene responses during nodulation [86]. *MtCEP1*-overexpressing plants phenocopy *skl* mutants [87]. Consistent with these observations, treatment with MtCEP1 counteracts the reduction in nodulation induced by ethylene [86] (Figure 2B).

Concluding Remarks and Future Perspectives

A variety of small peptides play roles in coordinating diverse aspects of plant root development, such as primary root meristem maintenance, protophloem formation during vascular development in the primary root, the systemic N-acquisition response, cell expansion during root growth, LR development under N limitation, LR formation, root hair formation, and root nodulation (summarized in Table 1). Although different classes of peptides play roles in different aspects of root development in different tissues, various other peptides are involved in the same developmental process, including LR development, indicating that crosstalk may take place between these peptides to properly regulate LR formation (see Outstanding Questions). Analysis of crystal structures of peptide–receptor complexes has revealed molecular recognition mechanisms for how peptides specifically bind to their cognate receptors, and also revealed the conserved structural features and structural differences of the receptors which can account for differential receptor–coreceptor complex formation [7,10,16,49,50]. CEPs and CLEs act as local signals for regulating LR formation under N limitation [42,44], and CEPs also act as long-distance signals to mediate the N-acquisition response in the primary root [32,33]. The CIF–GSO pair acts as a barrier surveillance system to guarantee sealing of the Casparian strip network in response to unfavorable mineral conditions [39–41]. Thus some small peptides are particularly adept in the plant adaptation response under adverse soil conditions.

Despite the extensive and growing knowledge on the roles of signaling peptide families in plant root development, the functions of many of these peptides are largely unknown. Because more than 1000 signaling peptide genes are predicted from the arabidopsis genome sequence [88,89], it is expected that new signaling peptides involved in root development will be identified. Moreover, only a small number of peptide–receptor pairs have been shown to play roles in plant root development. Combinations of biochemical assays, two-hybrid screening, genetic approaches, receptor kinase expression library screening and photoaffinity labeling, and structural analysis will be useful for identifying new peptide–receptor pairs [14–16]. CRISPR/Cas9-mediated gene targeting is an approach to overcome the limited number of loss-of-function peptide-encoding gene mutants available owing to their small gene size, and this approach was successfully used to generate a collection of mutants for CLE peptide-encoding genes [90]. Although the roles of many of the signaling peptides have been identified in plant root development, most of their downstream signaling pathways remain to be elucidated. Because signaling peptides control plant development, synergistic or antagonistic crosstalk most likely takes place between signaling peptides and conventional plant hormones, such as auxin, brassinosteroids, cytokinins, ethylene, gibberellins, and abscisic acid, that need further investigation, while some peptides and conventional plant hormones could act independently of each other to regulate particular aspects of plant development. The roles of peptides involved in plant responses to environmental changes in the root have been reported [32,33,40,42,43]. It will be of interest to investigate the function of signaling peptides that

Outstanding Questions

What are the molecular mechanisms by which the peptide signals perceived by LRR-RLKs are transduced to induce specific cellular responses, and what are their downstream signaling pathways?

How are the peptides directionally secreted or transported from where they are synthesized to other cells?

How do the signaling peptides coordinate developmental and environmental responses via cell-to-cell communication?

How do the peptides induce a distinct cellular response in a cell type-specific manner?

How are the peptide signaling pathways integrated into the conventional phytohormone signaling pathways during developmental and environmental responses?

How do hormone pathways crosstalk with peptide signaling in the root?

What other peptide–receptor pairs coordinate plant root development?

Are the functions and signaling mechanisms of the small peptides and their receptors identified in arabidopsis conserved in crops?

Table 1. Signaling Peptides, Receptors, and Their Functions in Plant Root Development

Peptides	Receptors	Coreceptors	Functions in root development	Species	Refs
CEP1	CEPR1, CEPR2		Root meristem activity, systemic N-acquisition response in primary root development, LR development	*Arabidopsis thaliana*	[31,32]
CEP3, CEP5, CEP9	CEPR1/XIP1, CEPR2		LR development	*Arabidopsis thaliana*	[31,43,44]
CIF1, CIF2	SGN3/GSO1, GSO2		Casparian strip formation	*Arabidopsis thaliana*	[38–40]
CLE1, CLE3, CLE4, CLE7	CLV1		N regulation of LR development	*Arabidopsis thaliana*	[42]
CLE26			Primary root growth, LR development, suppression of protophloem differentiation	*Arabidopsis thaliana*	[27,29]
CLE40	CLV1, ACR4		Differentiation of columella cells	*Arabidopsis thaliana*	[21–23]
CLE45	BAM3		Suppression of protophloem differentiation	*Arabidopsis thaliana*	[26,27].
GLV3, GLV6, GLV9			Root gravitropism	*Arabidopsis thaliana*	[17]
GLV4, GLV8			Root hair elongation	*Arabidopsis thaliana*	[52]
IDA	HAE, HSL2	SERK1, SERK2, BAK1/SERK3, SERK4	LR emergence	*Arabidopsis thaliana*	[61,62,64]
PSK	PSKR1, PSKR2	SERK1, SERK2, BAK1/SERK3	LR emergence and growth, cell elongation in the elongation/differentiation zone	*Arabidopsis thaliana*	[10,34,37,66–68]
PSY1	PSY1R		Cell elongation in the elongation/differentiation zone	*Arabidopsis thaliana*	[34]
RALF1, RALF19, RALF23	FER		LR initiation, root hair development	*Arabidopsis thaliana*	[56,57,69,72]
RALFL34			LR initiation	*Arabidopsis thaliana*	[58]
RGF1/GLV11, RGF2/GLV5, RGF3/GLV7	RGFRs/RGIs	SERK1, SERK2, SERK3/BAK1	Root stem cell maintenance, LR formation	*Arabidopsis thaliana*	[14,54]
TDIF/CLE41/CLE44	TDR/PXY	SERK1	LR formation, tracheary element differentiation	*Arabidopsis thaliana*	[46,48–50]
GmRIC1, GmRIC2, GmNIC	GmNARK		Root nodulation	*Glycine max*	[78,79]
LjCLE-RS1, LjCLE-RS2	HAR1, KLV		Root nodulation	*Lotus japonicus*	[80–83]
MtCEP1	CRA2		LR development, root nodulation	*Medicago truncatula*	[45]
MtCLE12, MtCLE13	SUNN		Root nodulation	*Medicago truncatula*	[84]

regulate plant root architecture during stress responses. The majority of studies on signaling peptides have been conducted on the model plant arabidopsis, with the exception of root nodulation and root–nematode interactions which have been investigated in *Medicago*, soybean, and pea. Peptide hormone studies need to be further extended to major crop plants such as rice and maize [91,92]. Current trends undoubtedly indicate that signaling peptides, their receptors, and the signaling pathways coordinating plant root development will be one of the major focuses of research in plant hormone biology in the coming years.

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Supplemental Information

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References

1. Pearce, G. *et al.* (1991) A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *Science* 253, 895–897
2. Czyzewicz, N. *et al.* (2013) Message in a bottle: small signalling peptide outputs during growth and development. *J. Exp. Bot.* 64, 5281–5296
3. Matsubayashi, Y. (2014) Posttranslationally modified small-peptide signals in plants. *Annu. Rev. Plant Biol.* 65, 385–413
4. Grienenberger, E. and Fletcher, J.C. (2015) Polypeptide signaling molecules in plant development. *Curr. Opin. Plant Biol.* 23, 8–14
5. Tavormina, P. *et al.* (2015) The plant peptidome: an expanding repertoire of structural features and biological functions. *Plant Cell* 27, 2095–2118
6. Lease, K.A. and Walker, J.C. (2006) The *Arabidopsis* unannotated secreted peptide database, a resource for plant peptidomics. *Plant Physiol.* 142, 831–838
7. Zhang, H. *et al.* (2016) Structural insight into recognition of plant peptide hormones by receptors. *Mol. Plant* 9, 1454–1463
8. Wu, Y. *et al.* (2016) Genome-wide expression pattern analyses of the *Arabidopsis* leucine-rich repeat receptor-like kinases. *Mol. Plant* 9, 289–300
9. Han, Z. *et al.* (2014) Structural insight into the activation of plant receptor kinases. *Curr. Opin. Plant Biol.* 20, 55–63
10. Wang, L. *et al.* (2015) Allosteric receptor activation by the plant peptide hormone phytosulfikine. *Nature* 525, 265–270
11. Brandt, B. and Hothorn, M. (2016) SERK co-receptor kinases. *Curr. Biol.* 26, R225–R226
12. Liebrand, T.W. *et al.* (2014) Two for all: receptor-associated kinases SOBIR1 and BAK1. *Trends Plant Sci.* 19, 123–132
13. Matsuzaki, Y. *et al.* (2010) Secreted peptide signals required for maintenance of root stem cell niche in *Arabidopsis*. *Science* 329, 1065–1067
14. Ou, Y. *et al.* (2016) RGF INSENSITIVE 1 to 5, a group of LRR receptor-like kinases, are essential for the perception of root meristem growth factor 1 in *Arabidopsis thaliana*. *Cell Res.* 26, 656–698
15. Shinohara, H. *et al.* (2016) Identification of three LRR-RKs involved in perception of root meristem growth factor in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 113, 3897–3902
16. Song, W. *et al.* (2016) Signature motif-guided identification of receptors for peptide hormones essential for root meristem growth. *Cell Res.* 26, 674–685
17. Whitford, R. *et al.* (2012) GOLVEN secretory peptides regulate auxin carrier turnover during plant gravitropic responses. *Dev. Cell* 22, 678–685
18. Ghorbani, S. *et al.* (2016) The SBT6.1 subtilase processes the GOLVEN1 peptide controlling cell elongation. *J. Exp. Bot.* 67, 4877–4887
19. Cock, J.M. and McCormick, S. (2001) A large family of genes that share homology with CLAVATA3. *Plant Physiol.* 126, 939–942
20. Whitford, R. *et al.* (2008) Plant CLE peptides from two distinct functional classes synergistically induce division of vascular cells. *Proc. Natl. Acad. Sci. U. S. A.* 105, 18625–18630
21. Stahl, Y. *et al.* (2009) A signaling module controlling the stem cell niche in *Arabidopsis* root meristems. *Curr. Biol.* 19, 909–914
22. Stahl, Y. *et al.* (2013) Moderation of *Arabidopsis* root stemness by CLAVATA1 and ARABIDOPSIS CRINKLY4 receptor kinase complexes. *Curr. Biol.* 23, 362–371
23. Guo, Y. *et al.* (2010) CLAVATA2 forms a distinct CLE-binding receptor complex regulating *Arabidopsis* stem cell specification. *Plant J.* 63, 889–900
24. Sarkar, A.K. *et al.* (2007) Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature* 446, 811–814
25. Depuydt, S. *et al.* (2013) Suppression of *Arabidopsis* protophloem differentiation and root meristem growth by CLE45 requires the receptor-like kinase BAM3. *Proc. Natl. Acad. Sci. U. S. A.* 110, 7074–7079
26. Rodriguez-Villalon, A. *et al.* (2014) Molecular genetic framework for protophloem formation. *Proc. Natl. Acad. Sci. U. S. A.* 111, 11551–11556
27. Rodriguez-Villalon, A. *et al.* (2015) Primary root protophloem differentiation requires balanced phosphatidylinositol-4,5-biphosphate levels and systemically affects root branching. *Development* 142, 1437–1446
28. Hazak, O. *et al.* (2017) Perception of root-active CLE peptides requires CORYNE function in the phloem vasculature. *EMBO Rep.* 18, 1367–1381
29. Czyewicz, N. *et al.* (2015) Modulation of *Arabidopsis* and monocot root architecture by CLAVATA3/EMBRYO SURROUNDING REGION 26 peptide. *J. Exp. Bot.* 66, 5229–5243
30. Czyewicz, N. and De Smet, I. (2016) The *Arabidopsis thaliana* CLAVATA3/EMBRYO-SURROUNDING REGION 26 (CLE26) peptide is able to alter root architecture of *Solanum lycopersicum* and *Brassica napus*. *Plant Signal. Behav.* 11, e1118598

31. Ohyama, K. *et al.* (2008) Identification of a biologically active, small, secreted peptide in *Arabidopsis* by *in silico* gene screening, followed by LC-MS-based structure analysis. *Plant J.* 55, 152–160
32. Tabata, R. *et al.* (2014) Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. *Science* 346, 343–346
33. Ohkubo, Y. *et al.* (2017) Shoot-to-root mobile polypeptides involved in systemic regulation of nitrogen acquisition. *Nat. Plants* 3, 17029
34. Amano, Y. *et al.* (2007) Tyrosine-sulfated glycopeptide involved in cellular proliferation and expansion in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 104, 18333–18338
35. Kwezi, L. *et al.* (2011) The phytosulfokine (PSK) receptor is capable of guanylate cyclase activity and enabling cyclic GMP-dependent signaling in plants. *J. Biol. Chem.* 286, 22580–22588
36. Hartmann, J. *et al.* (2014) Kinase activity and calmodulin binding are essential for growth signaling by the phytosulfokine receptor PSKR1. *Plant J.* 78, 192–202
37. Ladwig, F. *et al.* (2015) Phytosulfokine regulates growth in *Arabidopsis* through a response module at the plasma membrane that includes CYCLIC NUCLEOTIDE-GATED CHANNEL17, H^+-ATPase, and BAK1. *Plant Cell* 27, 1718–1729
38. Pfister, A. *et al.* (2014) A receptor-like kinase mutant with absent endodermal diffusion barrier displays selective nutrient homeostasis defects. *Elife* 3, e03115
39. Doblas, V.G. *et al.* (2017) Root diffusion barrier control by a vasculature-derived peptide binding to the SGN3 receptor. *Science* 355, 280–283
40. Nakayama, T. *et al.* (2017) A peptide hormone required for Casparian strip diffusion barrier formation in *Arabidopsis* roots. *Science* 355, 284–286
41. Alassimone, J. *et al.* (2016) Polarly localized kinase SGN1 is required for Casparian strip integrity and positioning. *Nat. Plants* 2, 16113
42. Araya, T. *et al.* (2014) CLE-CLAVATA1 peptide-receptor signaling module regulates the expansion of plant root systems in a nitrogen-dependent manner. *Proc. Natl. Acad. Sci. U. S. A.* 111, 2029–2034
43. Delay, C. *et al.* (2013) *CEP* genes regulate root and shoot development in response to environmental cues and are specific to seed plants. *J. Exp. Bot.* 64, 5383–5394
44. Roberts, I. *et al.* (2016) CEP5 and XIP1/CEPR1 regulate lateral root initiation in *Arabidopsis*. *J. Exp. Bot.* 67, 4889–4899
45. Imin, N. *et al.* (2013) The peptide-encoding *CEP1* gene modulates lateral root and nodule numbers in *Medicago truncatula*. *J. Exp. Bot.* 64, 5395–5409
46. Cho, H. *et al.* (2014) A secreted peptide acts on BIN2-mediated phosphorylation of ARFs to potentiate auxin response during lateral root development. *Nat. Cell Biol.* 16, 66–76
47. Ito, Y. *et al.* (2006) Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science* 313, 842–845
48. Kondo, Y. *et al.* (2014) Plant GSK3 proteins regulate xylem cell differentiation downstream of TDIF-TDR signalling. *Nat. Commun.* 5, 3504
49. Morita, J. *et al.* (2016) Crystal structure of the plant receptor-like kinase TDR in complex with the TDIF peptide. *Nat. Commun.* 7, 12383
50. Zhang, H. *et al.* (2016) Crystal structure of PXY–TDIF complex reveals a conserved recognition mechanism among CLE peptide–receptor pairs. *Cell Res.* 26, 543–555
51. Sun, Y. *et al.* (2013) Structure reveals that BAK1 as a co-receptor recognizes the BRI1-bound brassinolide. *Cell Res.* 23, 1326–1329
52. Kumpf, R.P. *et al.* (2013) Transcriptional and functional classification of the GOLVEN/ROOT GROWTH FACTOR/CLE-like signaling peptides reveals their role in lateral root and hair formation. *Plant Physiol.* 161, 954–970
53. Meng, L. *et al.* (2012) CLE-like (CLEL) peptides control the pattern of root growth and lateral root development in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 109, 1760–1765
54. Fernandez, A. *et al.* (2015) The GLV6/RGF8/CLEL2 peptide regulates early pericycle divisions during lateral root initiation. *J. Exp. Bot.* 66, 5245–5256
55. Murpy, E. and De Smet, I. (2014) Understanding the RALF family: a tale of many species. *Trends Plant Sci.* 19, 664–671
56. Atkinson, N.J. *et al.* (2013) Identification of genes involved in the response of *Arabidopsis* to simultaneous biotic and abiotic stresses. *Plant Physiol.* 162, 2028–2041
57. Bergonci, T. *et al.* (2014) *Arabidopsis thaliana* RALF1 opposes brassinosteroid effects on root cell elongation and lateral root formation. *J. Exp. Bot.* 65, 2219–2230
58. Murphy, E. *et al.* (2016) RALFL34 regulates formative cell divisions in *Arabidopsis* pericycle during lateral root initiation. *J. Exp. Bot.* 67, 4863–4875
59. Peret, B. *et al.* (2009) Lateral root emergence: a difficult birth. *J. Exp. Bot.* 60, 3637–3643
60. Butenko, M.A. *et al.* (2003) Inflorescence deficient in abscission controls floral organ abscission in *Arabidopsis* and identifies a novel family of putative ligands in plants. *Plant Cell* 15, 2296–2307
61. Kumpf, R.P. *et al.* (2013) Floral organ abscission peptide IDA and its HAE/HSL2 receptors control cell separation during lateral root emergence. *Proc. Natl. Acad. Sci. U. S. A.* 110, 5235–5240
62. Cho, S.K. *et al.* (2008) Regulation of floral organ abscission in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 105, 15629–15634
63. Santiago, J. *et al.* (2016) Mechanistic insight into a peptide hormone signaling complex mediating floral organ abscission. *eLife* 5, e15075
64. Meng, X. *et al.* (2016) Ligand-induced receptor-like kinase complex regulates floral organ abscission in *Arabidopsis*. *Cell Rep.* 14, 1330–1338
65. Schardon, P. *et al.* (2016) Precursor processing for plant peptide hormone maturation by subtilisin-like serine proteinases. *Science* 354, 1594–1597
66. Kutschmar, A. *et al.* (2009) PSK-alpha promotes root growth in *Arabidopsis*. *New Phytol.* 181, 820–831
67. Matsubayashi, Y. *et al.* (2002) An LRR receptor kinase involved in perception of a peptide plant hormone, phytosulfokine. *Science* 296, 1470–1472
68. Matsubayashi, Y. *et al.* (2006) Disruption and overexpression of *Arabidopsis* phytosulfokine receptor gene affects cellular longevity and potential for growth. *Plant Physiol.* 142, 45–53
69. Pearce, G. *et al.* (2001) RALF, a 5-kDa ubiquitous polypeptide in plants, arrests root growth and development. *Proc. Natl. Acad. Sci. U. S. A.* 98, 12843–12847
70. Morato do Canto, A. *et al.* (2014) Biological activity of nine recombinant AtRALF peptides: Implications for their perception and function in *Arabidopsis*. *Plant Physiol. Biochem.* 75, 45–54
71. Haruta, M. *et al.* (2014) A peptide hormone and its receptor protein kinase regulate plant cell expansion. *Science* 343, 408–411
72. Duan, Q. *et al.* (2010) FERONIA receptor-like kinase regulates RHO GTPase signaling of root hair development. *Proc. Natl. Acad. Sci. U. S. A.* 107, 17821–17826
73. Huang, G.Q. *et al.* (2013) *Arabidopsis* RopGEF4 and RopGEF10 are important for FERONIA-mediated developmental but not environmental regulation of root hair growth. *New Phytol.* 200, 1089–1101
74. Du, C. *et al.* (2016) Receptor kinase complex transmits RALF peptide signal to inhibit root growth in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 113, E8326–E8334
75. Berken, A. *et al.* (2005) A new family of RhoGEFs activates the Rop molecular switch in plants. *Nature* 436, 1176–1180
76. Gu, Y. *et al.* (2006) Members of a novel class of *Arabidopsis* Rho guanine nucleotide exchange factors control Rho GTPase-dependent polar growth. *Plant Cell* 18, 366–381

77. Li, C. *et al.* (2015) Glycosylphosphatidylinositol-anchored proteins as chaperones and co-receptors for FERONIA receptor kinase signaling in *Arabidopsis*. *Elife* 4, e06587
78. Reid, D.E. *et al.* (2011) Inoculation- and nitrate-induced CLE peptides of soybean control NARK-dependent nodule formation. *Mol. Plant Microbe Interact.* 24, 606–618
79. Lim, C.W. *et al.* (2011) Soybean nodule-enhanced CLE peptides in roots act as signals in GmNARK-mediated nodulation suppression. *Plant Cell Physiol.* 52, 1613–1627
80. Okamoto, S. *et al.* (2009) Nod factor/nitrate-induced *CLE* genes that drive HAR1-mediated systemic regulation of nodulation. *Plant Cell Physiol.* 50, 67–77
81. Soyano, T. *et al.* (2014) Nodule Inception creates a long-distance negative feedback loop involved in homeostatic regulation of nodule organ production. *Proc. Natl. Acad. Sci. U. S. A.* 111, 14607–14612
82. Okamoto, S. *et al.* (2013) Root-derived CLE glycopeptides control nodulation by direct binding to HAR1 receptor kinase. *Nat. Commun.* 4, 2191
83. Miyazawa, H. *et al.* (2010) The receptor-like kinase KLAVIER mediates systemic regulation of nodulation and non-symbiotic shoot development in *Lotus japonicus*. *Development* 137, 4317–4325
84. Mortier, V. *et al.* (2010) CLE peptides control *Medicago truncatula* nodulation locally and systemically. *Plant Physiol.* 153, 222–237
85. Osipova, M.A. *et al.* (2012) WUSCHEL-RELATED HOMEOBOX5 gene expression and interaction of CLE peptides with components of the systemic control add two pieces to the puzzle of autoregulation of nodulation. *Plant Physiol.* 158, 1329–1341
86. Mohd-Radzman, N.A. *et al.* (2016) Different pathways act downstream of the CEP peptide receptor CRA2 to regulate lateral root and nodule development. *Plant Physiol.* 171, 2536–2548
87. Xiao, T.T. *et al.* (2014) Fate map of *Medicago truncatula* root nodules. *Development* 141, 3517–3528
88. Carvalho-Ade, O. and Gomes, V.M. (2009) Plant defensins – prospects for the biological functions and biotechnological properties. *Peptides* 30, 1007–1020
89. Silverstein, K.A. *et al.* (2007) Small cysteine-rich peptides resembling antimicrobial peptides have been under-predicted in plants. *Plant J.* 51, 262–280
90. Yamaguchi, Y.L. *et al.* (2017) A collection of mutants for CLE-peptide-encoding genes in *Arabidopsis* generated by CRISPR/Cas9-mediated gene targeting. *Plant Cell Physiol.* 58, 1848–1856
91. Jin *et al.* (2016) *GAD1* encodes a secreted peptide that regulates grain number, grain length, and awn development in rice domestication. *Plant Cell* 28, 2453–2463
92. Sui, Z. *et al.* (2016) Overexpression of peptide-encoding *OsCEP6.1* results in pleiotropic effects on growth in rice (*O. sativa*). *Front. Plant Sci.* 7, 228
93. Hochholdinger, F. and Zimmermann, R. (2008) Conserved and diverse mechanisms in root development. *Curr. Opin. Plant Biol.* 11, 70–74
94. Benková, E. and Bielach, A. (2010) Lateral root organogenesis – from cell to organ. *Curr. Opin. Plant Biol.* 13, 677–683
95. Du, Y. and Scheres, B. (2018) Lateral root formation and the multiple roles of auxin. *J. Exp. Bot.* 69, 155–167
96. Grierson, C. and Schiefelbein, J. (2002) Root hairs. *Arabidopsis book* 1, e0060
97. Tominaga-Wada, R. *et al.* (2011) New insights into the mechanism of development of *Arabidopsis* root hairs and trichomes. *Int. Rev. Cell Mol. Biol.* 286, 67–106
98. Oka-Kira, E. and Kawaguchi, M. (2006) Long-distance signaling to control root nodule number. *Curr. Opin. Plant Biol.* 9, 496–502