Review

Exploring peptide hormones in plants:
identification of four peptide hormone-receptor pairs
and two post-translational modification enzymes

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Abstract: The identification of hormones and their receptors in multicellular organisms is one of the most exciting research areas and has lead to breakthroughs in understanding how their growth and development are regulated. In particular, peptide hormones offer advantages as cell-to-cell signals in that they can be synthesized rapidly and have the greatest diversity in their structure and function. Peptides often undergo post-translational modifications and proteolytic processing to generate small oligopeptide hormones. In plants, such small post-translationally modified peptides constitute the largest group of peptide hormones. We initially explored this type of peptide hormone using bioassay-guided fractionation and later by in silico gene screening coupled with biochemical peptide detection, which led to the identification of four types of novel peptide hormones in plants. We also identified specific receptors for these peptides and transferases required for their post-translational modification. This review summarizes how we discovered these peptide hormone–receptor pairs and post-translational modification enzymes, and how these molecules function in plant growth, development and environmental adaptation.

Keywords: secreted peptide, cell-to-cell communication, post-translational modification, Arabidopsis, ligand, receptor

1. Introduction

Cell-to-cell signaling mediated by hormones and membrane-localized receptors is one of the essential mechanisms by which the growth and development of multicellular organisms are regulated. Upon binding of such hormones to the extracellular domains of receptors, physicochemical interactions are converted into physiological outputs activating downstream signaling, which modulates cellular functions and fates through conformational changes in the receptors. Because membrane-localized receptors act as master switches of complex intracellular signaling processes, the identification of hormone–receptor pairs is one of the central issues of current biological research.

During the past 20 years, biochemical, genetic, and bioinformatic analyses have identified more than a dozen secreted peptide hormones and their receptors in plants.1)–4) These peptide hormone–receptor pairs have proven to be functionally more diverse than anticipated. Some of these peptides act as local signals during plant growth and development, whereas others are root-to-shoot long-distance signals required for environmental adaptation. The number of functionally characterized peptide hormones now exceeds the number of classical plant hormones.

Secreted peptide hormones can be divided into two major groups based on structural characteristics arising from their biogenesis pathways (Fig. 1). One major group of peptide hormones are small posttranslationally modified peptides characterized by the presence of post-translational modifications mediated by specific transferases and by their small size (approximately 5–20 amino acids) after proteolytic processing. The second group comprises cysteine-rich peptides characterized by the presence of an even number of cysteine residues (typically 6 or 8)
that participate in the formation of intramolecular disulfide bonds. In both cases, peptide hormone genes are initially translated as biologically inactive prepropeptides, followed by removal of the N-terminal signal peptide by a signal peptidase to afford a propeptide. Propeptides are further structurally modified by several enzymes to give biologically functional mature peptides.

My research career started with the purification of a chemical factor involved in the density effect of plant cell proliferation in vitro as a Ph.D. student at Nagoya University in the laboratory of Prof. Youji Sakagami. This bioassay-guided approach led to the identification of a peptide phytosulfokine (PSK), the first small post-translationally modified peptide hormone found in plants. Since then, I have been fascinated with the question of to what extent peptide signaling plays a role in plant growth and development. The major challenge in this research is, however, how to distinguish bona fide peptide hormones from the numerous unrelated peptides and protein fragments present in extracellular spaces. Additionally, because no one can predict the activities of undiscovered hormones, a conventional bioassay-guided approach is not applicable.

To this end, my group employed an in silico gene screening approach coupled with structural determination of mature peptides and receptor identification using a receptor expression library. This molecular-oriented strategy led to the identification of three peptide hormones, namely, C-terminally encoded peptide (CEP), involved in long-distance nitrogen demand signaling, root meristem growth factor (RGF), regulating root meristem development, and Casparian strip integrity factor (CIF) required for contiguous Casparian strip formation. These critical peptide hormones had long been overlooked, probably due to their gene redundancy. We also identified receptors involved in the perception of these peptide hormones and two important transferases required for post-translational modification of the hormones.

This review offers a personal overview of how we discovered these peptide hormone–receptor pairs and post-translational modification enzymes, and how these molecules contribute to plant growth and development. Information regarding other small post-translationally modified peptides and cysteine-rich peptides is reviewed elsewhere.

2. Novel approaches for the identification of peptide hormones and receptors in plants

2.1 In silico screening for peptide hormone candidates. After our identification of PSK and its family of precursor polypeptides by conventional bioassay-guided purification (described in section 3.1), we noticed several structural characteristics of the amino acid sequences within this family, as summarized in Fig. 2A. (a) These precursor polypeptides were approximately 100 amino acids in length and had N-terminal secretion signal sequences that can be detectable using public web-
based software. (b) The hormones (mature peptides) were encoded near the C-terminal region of the precursor. Moreover, amino acid sequences corresponding to the mature peptide domain were highly conserved within the family, but other domains exhibited low sequence conservation. This observation can be interpreted as functional mature peptide regions being under strong selective pressure and tending to exhibit higher sequence conservation than their neutral flanking regions. (c) The mature peptide was post-translationally modified. Because post-translational modifications such as sulfation and glycosylation require co-substrates that contain high-energy phosphate bonds, the biosynthesis of post-translationally modified peptides requires considerably more energy than the biosynthesis of other peptides. Nevertheless, post-translationally modified peptides have been evolutionarily conserved, suggesting that these modified peptides offer greater physiological benefit to plants than the energy cost for their biosynthesis. In this context, post-translational modifications can be indicative of hormones. (d) Genes encoding peptide hormones may exist as a family. The PSK family consists of five members in Arabidopsis and six in rice. These predictions were strengthened by the identification of additional peptide hormones such as the CLAVATA3/CLE peptide family, in which mature peptides are also encoded in the C-terminal domain, which is conserved among 32 members.

Based on this empirical rule, we hypothesized that if a family of secreted peptides in Arabidopsis shares a conserved domain near the C-terminus by in silico analysis and the conserved domain is indeed confirmed to be a part of the secreted mature peptide after post-translational modification by liquid chromatography-mass spectrometry (LC-MS)-based structural analysis (described in section 2.2), this family may encode functional peptide hormones. To test this idea, we performed computational screening of Arabidopsis genes encoding secreted polypeptide families that fulfilled the above criteria. We downloaded all Arabidopsis protein sequences (approx. 35,000) from the TAIR website and extracted potential families with primary translated products of approximately 100-amino-acid secreted polypeptides that share short, conserved domains near their C-terminus. This in silico screening enabled us to obtain at least three major functionally uncharacterized peptide families that we later found to be critical peptide hormones in plant growth and development.

2.2 LC-MS-based structural elucidation of mature peptides. Small peptide hormones are generated from precursor polypeptides by post-translational modification and proteolytic processing. Because such specific modification and trimming actions are critical for the activity of each peptide hormone, determination of the mature functional structures of the peptide hormones is indispensable for detailed analysis of peptide signaling.

To detect the mature peptide generated from target genes of interest, it is essential to extract apoplastic peptides lacking contamination with cytoplasmic molecules that hamper LC-MS-based structural analysis, even when the target peptide is overexpressed. To this end, we established a submerged culture system, in which Arabidopsis seeds are directly sown and grown in liquid culture medium. In these conditions, plants develop hyperhydric true leaves, which are characterized by a lack of cuticular wax formation. Hyperhydric leaves have vacuolated mesophyll cells with large intercellular spaces filled with water instead of air. Therefore, secreted peptides present in the intercellular apoplastic spaces diffuse into the culture medium without artificial manipulation. Peptides and proteins in the submerged culture medium can be extracted by phenol extraction followed by acetone precipitation, which effectively remove secondary metabolites and polysaccharides. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis of the resulting samples permitted highly reproducible and reliable identification of the mature peptide generated from each target gene expressed under the control of a constitutive promoter.

2.3 Receptor identification using receptor kinase expression library. Identification of the receptors for hormones is a key step to understand the molecular mechanisms underlying signal transduction. In addition, functional analysis of hormone signaling pathways from the receptor side is often an effective way to overcome genetic redundancy of the peptide ligands that frequently exist as multi-gene families in Arabidopsis. Two approaches may be applicable for receptor identification: biochemical binding assays using receptor proteins or hormone-insensitive assays using loss-of-function receptor mutants. We employed the former approach, because specific ligand binding activity is the most critical property of the receptors.

The Arabidopsis genome contains 625 receptor kinases (RKs) that are thought to function as membrane-localized receptors for extracellular
ligands including peptide hormones.\textsuperscript{15}) From the functional point of view, RKs can be divided into two groups, namely ligand-binding receptors, which directly interact with ligands, and co-receptors, which form heteromers with ligand-binding receptors to modulate signaling. Cumulative evidence suggests that the extracellular domain of ligand-binding receptors is quite large and mostly exceeds 400 amino acid residues. In addition, almost all genes encoding ligand-binding RKs contain no introns within the gene regions corresponding to the extracellular domain. Based on these empirical rules, we selected \(~100\) RKs that fulfill the above criteria and individually overexpressed them in conventional tobacco suspension cells to establish an RK expression library.\textsuperscript{7}) Photoaffinity labeling using photo-activatable derivatives of peptide hormones and screening of the RK expression library enabled us to identify peptide ligand–receptor pairs with high accuracy within a short period.\textsuperscript{8–10,16}

3. Peptide hormone-receptor pairs

3.1 Phytosulfokine and PSK receptor. PSK was identified by conventional bioassay-guided purification and provided an initial clue to small post-translationally modified peptide hormones in plants. Several structural characteristics of the amino acid sequences within PSK precursor polypeptides prompted the idea of searching for still-undiscovered peptide hormones by \textit{in silico} screening as described in section 2.1.

The relative growth rates of plant cells in suspension are often greatly affected by the initial cell population density. Dilution of isolated mesophyll cells of \textit{Asparagus officinalis} in excess culture medium suppressed cell division.\textsuperscript{5}) A similar phenomenon was also reported for transdifferentiation of mesophyll cells of \textit{Zinnia elegans} into xylem cells termed tracheary elements.\textsuperscript{17,18}) If \textit{Zinnia} mesophyll cells in suspension are cultured below a critical cell density, the frequency of tracheary element formation is greatly decreased. Somatic embryo formation from carrot cells in suspension also depends on cell density.\textsuperscript{19}) Of note, this density effect is alleviated by the addition of culture medium in which cells were previously grown. This circumstantial evidence suggests that a chemical factor secreted from individual cells affects the potential for cellular proliferation and differentiation.

We purified this growth-promoting signal from the culture medium using a bioassay system that assessed cell division activity of \textit{Asparagus} mesophyll cell suspensions incubated at low density, and identified a small peptide as a growth-promoting signal involved in the above-mentioned density effect in plant cell cultures of \textit{Asparagus}, carrot and \textit{Zinnia}.\textsuperscript{5}) This peptide is composed of only 5 amino acids including two sulfated tyrosine residues, and we named this factor PSK (Fig. 2A).\textsuperscript{5}) PSK was the first example of a small post-translationally modified peptide hormone in plants. \textit{In vitro} experiments showed that PSK has pleiotropic effects on cell growth and differentiation such as stimulating cell proliferation in \textit{Asparagus} and rice,\textsuperscript{5,20}) promoting tracheary element differentiation in \textit{Zinnia}\textsuperscript{17,21}) and enhancing somatic embryogenesis in carrot and Japanese cedar (\textit{Cryptomeria japonica}).\textsuperscript{19,22,23})

Based on cDNA cloning experiments, we demonstrated that PSK is produced from an approximately 80 amino acid precursor polypeptide via tyrosine sulfation and proteolytic processing.\textsuperscript{24,25}) Five paralogous PSK genes are present in the \textit{Arabidopsis} genome. PSK precursor polypeptides share a conserved domain close to the C-terminus, from which mature functional PSK peptides are generated (Fig. 2A). This domain structure of the PSK precursor polypeptides gave us a clue for an \textit{in silico} search for novel peptide hormones (described later). PSK genes are widely expressed in a variety of tissues in \textit{Arabidopsis}, and their expression is upregulated by wounding or interaction with microorganisms.\textsuperscript{24,26,27}) PSK homologs are present in both monocots and dicots as small gene families.\textsuperscript{28})

At the time we identified PSK peptide, little information was available on receptor candidates for peptide hormones in plants. Therefore, we searched for the PSK receptor using affinity purification. Initial assessment of PSK binding sites in several established cell lines suggested that rice Oc and carrot NC cells express a relatively high number of proteins binding PSK on their membranes.\textsuperscript{20,29,30}) Accordingly, we purified the PSK binding protein from solubilized membrane fractions of carrot NC cells using an affinity column on which PSK was immobilized. The carrot PSK receptor (PSKR) turned out to be a member of the leucine-rich repeat receptor kinase (LRR-RK) family,\textsuperscript{31}) which was later recognized as a major receptor family involved in the perception of peptide hormones.\textsuperscript{32}) The crystal structure of the PSK-PSKR complex was later resolved by Chai and colleagues.\textsuperscript{33}) We also found that \textit{Arabidopsis} has two PSKR orthologs, which we named AtPSKR1 and AtPSKR2; the former is the major component required for PSK perception.\textsuperscript{24})
Fig. 2. Structural characteristics of the primary amino acid sequences of precursor polypeptides that generate small post-translationally modified peptide hormones. Shown are the deduced amino acid sequences of the (A) PSK; (B) CEP; (C) RGF; and (D) CIF families.

**A**
- Phytosulfokine (PSK)
  - AAt1g3590 (PSK1)
  - AAt2g2860 (PSK2)
  - AAt3g4775 (PSK3)
  - AAt3g4780 (PSK4)
  - AAt5g5870 (PSK5)

**B**
- C-terminally encoded peptide (CEP)
  - AAt1g7445 (CEP1)
  - AAt3g3835 (CEP2)
  - AAt3g3440 (CEP3)
  - AAt3g5612 (CEP4)
  - AAt5g6815 (CEP5)
  - AAt5g6816 (CEP6)
  - AAt5g6818 (CEP10)
  - AAt5g6819 (CEP11)

**C**
- Root meristem growth factor (RGF)
  - AAt5g6010 (RGF1)
  - AAt1g3620 (RGF2)
  - AAt2g4802 (RGF3)
  - AAt3g3035 (RGF4)
  - AAt5g1541 (RGF5)
  - AAt5g1651 (RGF6)
  - AAt3g2240 (RGF7)
  - AAt3g3830 (RGF8)
  - AAt5g4770 (RGF9)
  - AAt3g4605 (RGF10)

**D**
- Caspian strip integrity factor (CIF)
  - AAt2g6385 (CIF1)
  - AAt4g3680 (CIF2)
  - AAt4g3610 (CIF3)
  - AAt4g3640 (CIF4)
  - AAt4g3610 (CIF5)
  - AAt4g3640 (CIF6)
  - AAt4g3610 (CIF7)
  - AAt4g3640 (CIF8)
  - AAt4g3610 (CIF9)

**C-terminally encoded peptide (CEP)**
- AAt1g7445 (CEP1)
  - AAt3g3835 (CEP2)
  - AAt3g3440 (CEP3)
  - AAt3g5612 (CEP4)
  - AAt5g6815 (CEP5)
  - AAt5g6816 (CEP6)
  - AAt5g6818 (CEP10)
  - AAt5g6819 (CEP11)

**Asp Phe Arg Hyp Thr Asn Pro Gly Asn Ser Hyp Gly Val Gly His**
The *pskr1* mutant exhibits premature senescence and gradually loses the potential to form callus as tissue matures. This mutant was later shown to be defective in innate plant immune responses triggered upon the perception of elicitors released by pathogens, in root and hypocotyl elongation, and in *Agrobacterium*-induced tumor growth (reviewed in 27)). Thus, available evidence indicates that PSK signaling affects cellular longevity and potential for growth, and thereby exerts a pleiotropic effect on growth and development in response to environmental conditions.

### 3.2 C-terminally encoded peptide and CEP receptor.

The above-mentioned in silico gene screening identified at least three families of peptide hormone candidates that contain family-specific conserved domains near the C-terminus (Fig. 2B–D). We determined that one of these candidate genes encodes a polypeptide that generates a 15 amino acid mature peptide with two hydroxyproline (Hyp) residues, and named it and its homologs the CEP family after their structural characteristics (Fig. 2B). A total of 15 CEP family genes have been found in the *Arabidopsis* genome. CEP genes are present in both dicots and monocots but are absent in lower land plants such as moss and green algae. Expression of CEP genes is mainly found in vascular tissues of the lateral roots, suggesting a role in roots, but severe genetic redundancy obscured the functions of the CEPs at that time.

We overcame genetic redundancy on the ligand side by identifying receptors for CEP by exhaustive photoaffinity labeling using the RK expression library. In contrast to multiple redundancy on the ligand side, only two RKs were found for the CEP receptor, which we named CEPR1 and CEPR2. These two RKs belong to LRR-RK subfamily XI. Expression of the major receptor CEPR1 was not limited to roots but rather was predominantly detected in the vascular veins of cotyledons and mature leaves. The receptor double mutant ultimately revealed loss-of-function phenotypes of CEP signaling, which were characterized by pale-green leaves, enhanced lateral root elongation, and shorter stems accompanied by anthocyanin accumulation. These phenotypes are reminiscent of typical responses to nitrogen (N) starvation, and indeed were accompanied by a considerable reduction in the expression of high-affinity nitrate transporter gene *NRT2.1* in roots.

These spatially distant expression patterns of CEP family peptide ligands and the receptor CEPR1, together with loss-of-function phenotypes similar to N starvation responses, suggested that the CEP–CEPR system may be involved in long-distance signaling regulating N acquisition. Related to this, it was first reported in the 1970s that N starvation of a portion of the root system in a heterogeneous soil N environment can upregulate nitrate uptake in a distant part of the roots exposed to a N-rich medium, compensating for N deficiency. This long-distance systemic response was postulated to be controlled by a N-demand signal emitted from the N-starved roots and ultimately led to the idea that CEP family peptides act as the long-sought N-demand signal (Fig. 3A, magenta arrow).

Based on this hypothesis, we analyzed whether the CEP–CEPR system is involved in long-distance N-demand signaling by studying CEP expression patterns in a N-starved environment, together with *cepr* mutant phenotypes in split-root conditions, in which the root system is separated into two parts exposed to different nutrient conditions (Fig. 3B). We also examined CEP translocation from roots to shoots, and finally concluded that this ligand–receptor pair is a critical component for the first half of the systemic N-demand signaling pathway (Fig. 3A). CEP family peptides are induced in the portion of the root system directly experiencing N starvation and act as root-derived ascending N-demand signals transported through the xylem to the leaves (Fig. 3A, magenta arrow), where they are recognized by CEPR localized on the phloem side in the vasculature of the leaves. Perception of CEP by CEPR triggers the production of a shoot-derived descending signal (described later) that upregulates nitrate transporter genes in the distant part of the roots to compensate for local N deficiency.

Identification of the ascending CEP family peptides raised the question of the molecular identity of the descending shoot-derived secondary signal. We searched for this signal by mechanically isolating vascular tissues, in which CEP is expressed, from wild-type, CEP-treated, and *cepr* mutant plants, followed by transcriptome analysis using a microarray system. This approach identified several CEP-inducible genes that encode small phloem-specific polypeptides. We found that, when overexpressed, two of them led to upregulation of the nitrate transporter gene *NRT2.1* in roots and accordingly named them CEP downstream (CEPD) polypeptides. CEPDs are approximately 100 amino acid polypeptides that belong to a plant-specific, non-secreted peptide family. Of note, although *CEPD*...
genes are expressed exclusively in leaf phloem, CEPD polypeptides are detected in the root vascular region, indicating that CEPDs function as shoot-to-root mobile signals (Fig. 3A, blue arrow).

Our final question was how CEPD polypeptides induce NRT2.1 expression, specifically on the side of the root system exposed to the N-rich medium under heterogeneous N conditions. One possibility was that shoot-derived CEPD polypeptides are selectively translocated to the root system of the N-rich side. This possibility was, however, ruled out by the observation that there was no apparent difference in the abundance of CEPDs in the roots even when one side of the root system was starved of N. The other possibility was that CEPDs are distributed to both sides of the root system but activate NRT2.1 expression only in roots where nitrate is available. We tested this possibility using CEPD1-overexpressing plants and found that NRT2.1 expression was specifically induced in roots where nitrate is available. We tested this possibility using CEPD1-overexpressing plants and found that NRT2.1 expression was specifically induced in roots exposed to the N-rich medium but not in N-starved roots of the split-root system. Thus, shoot-derived CEPD polypeptides upregulate NRT2.1 expression in roots specifically when nitrate is present in the soil, thereby compensating for local N deficiency at the whole-plant level.40)

Plants, as sessile organisms, continuously face a complex array of environmental fluctuations. Our findings demonstrated that although plants do not have a circulation system, like the heart and circulatory system in animals, they have evolved sophisticated long-distance signaling mechanisms to respond to fluctuating environments using xylem-mobile ascending peptides as “hunger” signals and phloem mobile descending peptides as “nutrient intake-facilitating” signals (Fig. 3A).42) These fundamental mechanistic insights should provide a conceptual framework for understanding systemic long-distance root-to-shoot-to-root signaling in plants.

3.3 Root meristem growth factor and RGF receptor. The initial clue that led to the discovery of the RGF peptide family came from the identification of Arabidopsis tyrosylprotein sulfotransferase (TPST) (described in section 4.1,11)), a post-translational modification enzyme required for tyrosine sulfation. Because TPST is a single-copy gene, the phenotype of a loss-of-function mutant of Arabidopsis TPST (tpst-1) reflects a deficiency in the biosynthesis of all functional tyrosine-sulfated peptides. Of note, tpst-1 shows an extremely short-root phenotype accompanied by a considerable decrease in proximal meristem activity in the root apical meristem,11,43) suggesting that at least one tyrosine-sulfated peptide regulates root meristem development. Because proximal meristem activity of tpst-1 was not recovered by treatment with known sulfated peptides such as PSK, we predicted that an as yet undiscovered sulfated peptide was indispensable for root meristem development.

We searched for relevant tyrosine-sulfated peptides by phenotypic rescue experiments using synthetic sulfated peptides nominated by in silico gene screening coupled with LC-MS-based structural determination. As stated in the previous section, in silico gene screening identified at least three families of peptide hormone candidates that contain...
family-specific conserved domains near the C-terminus. Notably, two of them encoded tyrosine-sulfated peptides and thus represent strong candidates for novel hormones.

We found that one of these sulfated peptide families indeed rescued proximal meristem activity of \textit{tpst-1} at nanomolar concentrations and accordingly named it the RGF family\(^{9}\) (Fig. 2C). Mature RGF1 is a 13 amino acid sulfated peptide derived from the C-terminal conserved domain of the precursor polypeptides; in \textit{Arabidopsis}, 9 members (later found to be 11\(^{14}\)) belong to this group. RGF1 is specifically expressed in quiescent center cells and columellar stem cells in the root tip.\(^{9}\) An additional 4 RGF family members are also expressed in the root stem cell area, suggesting redundant roles in the root apical meristem. Indeed, \textit{rgf1} \textit{rgf2} \textit{rgf3} triple mutants show a short-root phenotype characterized by a decrease in the number of meristematic cells.\(^{9}\) Because the C-terminal sequence of CLE18 is similar to RGF1, some researchers later named the RGF family the “CLE-like” family.\(^{45}\) Additionally, because overexpression of RGF family peptides often causes irregular root waving (“golven” in Dutch), some researchers refer to this as the GOLVEN family.\(^{46}\) RGF genes are also found in the rice and poplar genomes, suggesting evolutionarily conserved roles of RGF across the plant kingdom.\(^{46}\)

We identified receptors for RGF using a custom-made RK expression library. Exhaustive photoaffinity labeling revealed that 3 LRR-RKs in subfamily XI specifically interact with RGF family peptides.\(^{7}\) We observed a considerable decrease in meristematic cell number in the triple mutant to a level similar to that of the \textit{tpst-1} mutant, accompanied by insensitivity to RGF, and named these receptor proteins RGFR1, RGFR2, and RGFR3. RGFR1 and RGFR2 are predominantly expressed in the proximal meristem including the elongation zone and gradually decrease in expression towards the differentiation zone. In contrast, RGFR3 promoter activity is detected in the more basal region of the elongation zone and the differentiation zone. Unexpectedly, RGFR2 is also known as \textit{ROOT CLAVATA-HOMOLOG1} (\textit{RCH1}); its promoter is often used to express transgenes specifically in the root meristem.\(^{47}\) No one, however, had noticed the fundamental functions of this receptor family due to functional redundancy until our analysis focused on direct ligand binding properties. The crystal structure of the RGF1-RGFR1 complex was later resolved by Chai and colleagues.\(^{48}\)

We also uncovered that PLETHORA1 (PLT1) and PLT2 transcription factors are very proximal molecular targets of RGF signaling. PLT genes, which are specifically expressed in the stem cell area in the root meristem, encode AP2-domain transcription factors that mediate patterning of the root stem cell niche.\(^{49}\) PLT proteins display a gradient distribution, with maximal distribution in the stem cell area, and this gradient is essential for maintenance of the root stem cell niche and transit-amplifying cell proliferation.\(^{50}\) However, the fundamental molecule that defines this gradient has been elusive.

In wild-type seedlings, PLT proteins show gradients that extend into the region of transit-amplifying cells or the elongation zone. However, in the \textit{rgf1} \textit{rgf2} \textit{rgf3} ligand triple mutant, or \textit{rgfr1} \textit{rgfr2} \textit{rgfr3} receptor triple mutant, PLT gradient dimensions were considerably reduced. Conversely, exogenous application of RGF to the \textit{rgf1} \textit{rgf2} \textit{rgf3} mutant causes drastic enlargement of the PLT expression domain in the mutant to levels even higher than in wild-type plants. Importantly, because externally added RGF1 restores PLT protein expression patterns without major changes in PLT gene expression, this response is not at the transcriptional level but rather at the protein level, possibly through the stabilization of PLT proteins.

Collectively, RGF peptides that are secreted from the stem cell region create a diffusion-based concentration gradient extending shootward from the stem cell area in roots\(^{9}\) (Fig. 4). This RGF peptide gradient is reflected as a PLT protein gradient by the membrane receptors, RGFRs, and allows correct pattern formation in the proximal meristem. Despite its emerging importance, RGF signaling has been overlooked due to the high degree of genetic redundancy both in ligands and receptors. The gradient of RGF is defined by the diffusion coefficient of the peptides, which is a physical parameter solely proportional to the molecular weight, independent of environmental fluctuations. Regulation of PLT expression patterns by a simple diffusion-based RGF peptide gradient is a sophisticated system to ensure robust root growth and development in fluctuating natural environments.

3.4 Casparian strip integrity factor and CIF receptor. Because nutrients often accumulate against a concentration gradient in the root xylem vessels, vascular plants have evolved a physical barrier that prevents passive apoplastic diffusion of ions and water across the endodermal cells surround-
ing the vascular bundles. This hydrophobic barrier that seals the extracellular spaces between neighboring endodermal cells is called the Casparian strip (Fig. 5). The third peptide hormone candidate family that we identified by in silico screening was found to act in Casparian strip formation, because this peptide family binds directly to an LRR-RK, GASSHO1 (GSO1)/SCHENGEN3 (SGN3), which regulates the integrity of the Casparian strip.

*Arabidopsis* has two peptides that belong to this peptide family (Fig. 2D). We determined that their mature structures are 21 amino acid long and contain one residue of sulfated tyrosine and two residues of Hyp. One peptide is expressed in the stele, especially at the phloem pole, of the mature region of the primary roots, and the other one is in the root stele in the elongation and differentiation zones of both primary and lateral roots. We also found by exhaustive photoaffinity labeling that this peptide family directly binds to GSO1/SGN3 and its closest homolog GSO2. Importantly, it has been reported that GSO1/SGN3 is expressed in root endodermal cells and its loss-of-function mutations result in the formation of a repeatedly interrupted, discontinuous Casparian strip. These findings indicated that these two sulfated peptides act as ligands for GSO1/SGN3 and GSO2 receptors to regulate contiguous Casparian strip formation in roots.

We confirmed that the double mutant devoid of these two peptide genes was a phenocopy of the receptor mutant, and external application of the synthetic peptides restored contiguous Casparian strip formation in the mutant roots. Therefore, we called this family of peptides CIF. Because CIF is a tyrosine-sulfated peptide, the *tpst-1* mutant, which lacks tyrosine sulfation enzymes, is also defective in Casparian strip formation. Independently, Geldner and colleagues identified *tpst-1* in the course of

![Fig. 4. RGF family peptides regulate root meristem development through the PLT pathway. (A) Schematic representation of the *Arabidopsis* root. (B) Whole-mount *in situ* hybridization of RGF1 mRNA. RGF genes are specifically expressed in the stem cell region in the root tip. (C) Whole-mount immunostaining of wild-type roots using an anti-RGF1 antibody. RGF peptides create a diffusion-based concentration gradient extending shootward from the stem cell area in roots. (D) Root meristem of wild-type seedling expressing PLT2-GFP. The RGF peptide gradient is reflected as a PLT transcription factor protein gradient and allows correct pattern formation in the proximal root meristem. Images B and C are adapted from our previous report.](image)

![Fig. 5. Schematic cross-section of roots showing the Casparian strip. The hydrophobic barrier that seals the extracellular apoplastic space between neighboring endodermal cells is called the Casparian strip (shown in red). The Casparian strip prevents passive apoplastic diffusion of ions and water across the endodermal cells (solid blue arrows). Essential mineral ions move across the endodermis by symplastic transport (dashed blue arrows). CIF peptides expressed in the stele are required for contiguous Casparian strip formation. This illustration is modified from a figure in a previous report.](image)
and specifically identified CIF peptides by phenotyping rescue experiments using synthetic sulfated peptides selected by in silico gene screening.\(^{53}\) His group further identified CIF peptides by phenotypic rescue experiments using synthetic sulfated peptides selected by in silico gene screening.\(^{53}\)

The Casparian strip plays important roles in environmental adaptation by acting as a physical barrier that prevents unfavorable leakage of ions between the xylem and the soil.\(^{54}\) In nutrient-limiting conditions such as low potassium, the cif1-1 cif2-1 double mutant exhibits potassium deficiency symptoms that are far more severe than in wild-type plants. Our xylem sap analysis revealed that the potassium level in the xylem sap of the mutant was lower than that of the wild-type, due to concentration-dependent outward leakage.\(^{10}\) We also found that the cif1-1 cif2-1 mutant is highly sensitive to excess iron. At normal iron concentrations, the iron content in the xylem sap of the cif1-1 cif2-1 mutant was virtually the same as that of the wild-type. In contrast, the iron level in the xylem sap of the cif1-1 cif2-1 mutant cultured with excess iron was considerably higher than the wild-type, probably due to inward leakage. Thus, Casparian strip mutants are defective in ion homeostasis in the xylem because of inward or outward leakage of ions depending on the ionic concentration gradient across the endodermal cell layer, leading to pleiotropic phenotypes in unfavorable mineral environments.

4. Post-translational modification enzymes

Secreted peptides move from cell to cell through the extracellular apoplastic space solely by simple diffusion, with the diffusion coefficient dependent on molecular size. Because smaller molecular size leads to higher diffusion rates, shorter oligopeptides are theoretically advantageous in cell-to-cell signaling over larger proteins. Fewer residues in the peptide chain, however, results in lower structural diversity and specificity. To solve this paradox, multicellular organisms likely evolved a post-translational modification system that alters the physicochemical properties of peptides by changing their net charge or conformation. Indeed, post-translational modification is often critical for the physiological function and specific receptor binding activity of small peptide hormones. To date, three types of post-translational modifications have been found in peptide hormones in plants: tyrosine sulfation, proline hydroxylation, and hydroxyproline arabinosylation (reviewed in 55)). We identified key enzymes for tyrosine sulfation and hydroxyproline arabinosylation by affinity purification.\(^{11,12}\)

4.1 Tyrosine sulfation. Tyrosine sulfation is a post-translational modification occasionally found in peptides and proteins synthesized through the secretory pathway both in plants and animals.\(^{56}\) A specific enzyme involved in this modification was first identified in mice and humans and named tyrosyl-protein sulfotransferase (TPST).\(^{57,58}\) Animal TPST is a type II membrane protein that has a large C-terminal catalytic domain oriented in the lumen of the Golgi and a single transmembrane domain near the N-terminus. After public release of the mouse TPST sequence, many groups, including ours, searched for its plant counterpart by BLAST analysis, but no ortholog was identified in the Arabidopsis protein database. This suggested that plants evolved plant-specific TPSTs with a primary structure distinct from that of animals.

In general, TPST catalyzes the transfer of a sulfate from a donor 3’-phosphoadenosine 5’-phosphosulfate to the phenolic group of tyrosine within the acceptor peptide by forming an enzyme-substrate ternary complex (Fig. 6A). Therefore, we immobilized the acceptor peptide on a column in an attempt to purify plant TPST from solubilized Arabidopsis membrane fractions by affinity chromatography. However, the initial trial using PSK precursor peptide was unsuccessful, probably due to insufficient affinity for purification.

Another chance for affinity purification of TPST arose from the identification of PSY1, the second tyrosine-sulfated peptide in plants.\(^{29}\) This peptide was found by peptidomics analysis in a highly acidic fraction obtained by ion-exchange chromatography of the culture medium of Arabidopsis cells. PSY1 is an 18 amino acid peptide that exhibits cell division stimulatory activity similar to that of PSK. We again immobilized the PSY1 precursor peptide on a column and attempted to purify Arabidopsis TPST. Fortunately, this PSY1 column specifically adsorbed TPST activity, allowing enrichment of TPST protein to a level detectable by SDS-PAGE. It has been reported that multiple acidic amino acids near the tyrosine residue, as is the case for PSY1, significantly enhance sulfation,\(^{60}\) and thereby likely increases affinity for TPST.

Arabidopsis TPST (AtTPST) was identified to be a 62-kD transmembrane protein localized in the cis-Golgi.\(^{11}\) As we hypothesized, AtTPST showed no sequence similarity with animal TPST and, more surprisingly, was a type I membrane protein, which has a transmembrane domain near the C-terminus and a large luminal catalytic domain toward the
N-terminus, the opposite topological orientation compared with animal TPST. Plants and animals are likely to have independently acquired enzymes for tyrosine sulfation through convergent evolution. Nonetheless, AtTPST has a subtle footprint of a sulfotransferase near the C-terminus sharing similarity with heparan sulfate 6-O-sulfotransferase 2 (HS6ST2). It should be noted that AtTPST had been registered in the Arabidopsis protein database before our discovery as a shorter protein lacking the HS6ST2 domain due to misassignment of the splicing site. This is probably why AtTPST escaped gene hunting by bioinformaticians for such a longer period.

AtTPST is expressed throughout the plant, and the highest expression is in the root apical meristem. A knockout mutant of AtTPST (tpst-1) displayed pale green leaves and early senescence in the above-ground tissues and had an extremely short root phenotype accompanied by a considerable decrease in proximal meristem activity. Because AtTPST is a single-copy gene, the phenotype of the tpst-1 mutant should reflect the deficiency in the biosynthesis of all functional tyrosine-sulfated peptides. Indeed, root defects associated with tpst-1 were explained later as being caused by loss of sulfation on RGF peptides that are required for maintenance of the root stem cell niche. It has also been reported that the tpst-1 mutant showed hypersensitivity to copper deficiency and defective formation of the Casparian strip in roots. A recent discovery of a sulfated CIF peptide that is indispensable for Casparian strip formation explains this phenotype well.

4.2 Hyp arabinosylation. Proline residues in secreted peptides and proteins are often hydroxylated to Hyp, a post-translational modification found both in plants and animals. In plants, Hyp residues are occasionally modified further with three residues of L-arabinose, giving rise to Hyp O-triarabinoside. This Hyp O-arabinosylation was originally discovered in extracellular structural proteins such as extensins, but later found in several peptide hormones.

The occurrence of pentose residues in peptide hormones was initially suggested in the course of mass spectroscopic analysis of a defense peptide, TobHypSys, but the molecular nature of the sugar moiety remained elusive. We detected pentose sugars also in growth-promoting peptide PSY1 and determined that the sugar moiety consisted of three residues of L-arabinose.

The first structurally characterized Hyp O-arabinosylated peptide hormone was CLV3, which has been shown to play a definitive role in regulating stem cell populations in the shoot and floral meristems of Arabidopsis. We detected Hyp...
O-arabinosylated CLV3 in the medium derived from a submerged culture of *Arabidopsis* plants overexpressing CLV3 during an exhaustive peptidomics search, and determined by gas chromatography–mass spectrometry (GC-MS)-based linkage analysis that one of the Hyp residues is modified with β-1,2-linked triarabinoside. We also chemically synthesized arabinosylated CLV3 glycopeptide by intramolecular aglycone delivery and found that Hyp O-arabinosylation enhanced both biological activity and receptor binding activity by means of conformational alterations within the peptide backbone.66)

The physiological importance of Hyp O-arabinosylation in peptide hormones was further demonstrated by the discovery of the *CLE-RS2* gene, which controls the number of root nodules to achieve balance in the symbiotic relationship between leguminous plants and rhizobia.67) This symbiosis enables nitrogen fixation in the nodules and is beneficial to the host plants; however, excessive nodule formation is deleterious to the plants because the energy cost outweighs the need for fixed nitrogen. The *CLE-RS2* gene is upregulated in roots upon rhizobial infection and suppresses excessive nodule formation via a negative feedback loop. We determined that mature *CLE-RS2* is a 13 amino acid peptide, in which one of the Hyp residues is modified with triarabinoside.68) The physiological importance of arabinosylation of *CLE-RS2* is more pronounced than that of CLV3, as the arabinosyl chain is indispensable for both biological activity and receptor binding activity.

Biosynthesis of Hyp triarabinoside involves two distinct steps, namely initial attachment of L-arabinose to Hyp and successive elongation of the L-arabinose chain. The first enzyme, designated hydroxyproline arabinosyltransferase (HPAT) (Fig. 6B), has been predicted to exist in plants since the discovery of extensins in the 1960s, but its molecular identity remained elusive for many years.

Making use of our experience in the purification of TPST, we purified HPAT from solubilized *Arabidopsis* membrane fractions by affinity chromatography and identified 42 kD Golgi-localized type II transmembrane proteins that contained a single transmembrane domain near the N-terminus.12) Notably, purified *Arabidopsis* HPAT1 and HPAT2 showed no sequence similarity to any known functionally characterized proteins, which is probably why HPAT escaped similarity-based searches for glycosyltransferases. BLAST analysis showed that *Arabidopsis* has one additional HPAT family protein (HPAT3), resulting in a total of three members. A recent computational analysis of these sequences suggested that they may be conformationally similar to the glycosyltransferase GT8 family.69)

Our identification of *Arabidopsis* HPAT family arabinosyltransferases shed light on the function of their orthologs in leguminous plants such as *Pisum sativum* NOD3 and *Medicago truncatula* RDN1, whose loss-of-function mutants show hypernodulation phenotypes.70) Although their enzymatic activities have yet to be confirmed, the fact that NOD3 and RDN1 belong to the HPAT family strongly supports our previous findings that Hyp O-arabinosylated CLE-RS2 peptide induced by rhizobia acts as an autoregulation signal to suppress excess nodulation.68)

Similarly, tomato FIN, whose loss-of-function mutants show fasciated flowers associated with enlarged shoot apical meristems, has also been proved to be an HPAT family protein.71) A collaborative team including our group found that this phenotype was fully rescued by the in vitro treatment of fin mutant apices with a synthetic Hyp O-arabinosylated tomato CLV3 peptide.71) Thus, it is likely that loss of FIN caused under-arabinosylation of tomato CLV3 and led to downregulation of the CLV signaling pathway, resulting in overaccumulation of stem cells in the shoot apical meristems. However, this meristem phenotype caused by loss of HPAT has not been reported in plant species other than tomato, suggesting that the extent of contribution of Hyp O-arabinosylation to CLV3 function differs between species.72)

Because Hyp O-arabinosylation is also required for cell wall structural proteins, loss-of-function mutations in *HPAT* genes in *Arabidopsis* cause pleiotropic phenotypes that include enhanced hypocotyl elongation, defects in cell wall thickening, early flowering, early senescence, and defective growth of pollen tubes, causing a transmission defect through the male gametophyte.12) Detailed phenotypic analyses of *hpat* mutants will provide a more complete picture of how Hyp O-arabinosylated glycoproteins and glycopeptides contribute to plant growth and development.

5. Future directions

Numerous research efforts including our own have identified more than a dozen secreted peptide hormones in plants. Over the past decade, the number of functionally characterized peptide signals has increased several-fold and now exceeds the number of classical plant hormones such as auxin
and gibberellin. Nonetheless, many genes encoding possible secreted peptides remain in the *Arabidopsis* genome. In the TAIR10 protein data set, as many as 1,086 genes encode potential secreted peptides (SignalP score $> 0.75$) of between 50 and 150 amino acid residues. Continued efforts should be made to further identify peptide hormones and explore how they function during plant growth and development.

Previously, peptide hormones were identified using classical bioassay-guided purification. The feasibility of this approach, however, largely depends on the quality and sensitivity of the bioassay system and the abundance of the peptides in the samples. In addition, one paradoxical problem is that, because no one knows the activities of undiscovered molecules, it is not possible to establish a bioassay system aimed at the detection of novel hormones. Indeed, no additional peptide hormones have been identified by bioassay-guided purification since 2006. Similarly, no peptide hormones have been identified using a forward genetics approach since 2003, indicating that the non-redundant peptide genes that produce a visible phenotype upon mutation have already been fully characterized. Thus, alternative strategies, such as genomics- or transcriptomics-based *in silico* approaches, preferably coupled with biochemical and molecular biology techniques, offer promise for further research into peptide hormones in plants.

In plants, peptide hormones have long been thought to exclusively mediate short-distance cell-to-cell signaling involved in growth and development. Cumulative examples including our CEP-CEPR system, however, have provided a new functional model in which peptides respond to environmental stimuli and mediate long-distance communication. Plants may employ various types of peptide signals for organ-to-organ communication and adapt to diverse and complex environmental stresses more dynamically and ingeniously than previously assumed.

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References

1) Matsubayashi, Y. (2014) Posttranslationally modified small-peptide signals in plants. Annu. Rev. Plant Biol. 65, 385–413.
2) Grienengerber, E. and Fletcher, J.C. (2015) Polypeptide signaling molecules in plant development. Curr. Opin. Plant Biol. 23, 8–14.
3) Endo, S., Betsuyaku, S. and Fukuda, H. (2014) Endogenous peptide ligand-receptor systems for diverse signaling networks in plants. Curr. Opin. Plant Biol. 21, 140–146.
4) Marshall, E., Costa, L.M. and Gutierrez-Marcos, J. (2011) Cysteine-rich peptides (CRPs) mediate diverse aspects of cell-cell communication in plant reproduction and development. J. Exp. Bot. 62, 1677–1686.
5) Matsubayashi, Y. and Sakagami, Y. (1996) Phyto-sulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of *Asparagus officinalis* L. Proc. Natl. Acad. Sci. U.S.A. 93, 7623–7627.
6) Ohyama, K., Ogawa, M. and 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. Plant J. 55, 152–160.
7) Shinohara, H., Mori, A., Yasue, N., Sumida, K. and Matsubayashi, Y. (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.
8) Tabata, R., Sumida, K., Yoshii, T., Ohyama, K., Shinohara, H. and Matsubayashi, Y. (2014) Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. Science 346, 343–346.
9) Matsuzaki, Y., Ogawa-Ohnishi, M., Mori, A. and Matsubayashi, Y. (2010) Secreted peptide signals required for maintenance of root stem cell niche in *Arabidopsis*. Science 329, 1065–1067.
10) Nakayama, T., Shinohara, H., Tanaka, M., Baba, K., Ogawa-Ohnishi, M. and Matsubayashi, Y. (2017) A peptide hormone required for Casparian strip diffusion barrier formation in *Arabidopsis* roots. Science 355, 284–286.
11) Komori, R., Amano, Y., Ogawa-Ohnishi, M. and Matsubayashi, Y. (2009) Identification of tyrosyl-protein sulfotransferase in *Arabidopsis*. Proc. Natl. Acad. Sci. U.S.A. 106, 15067–15072.
12) Ogawa-Ohnishi, M., Mitsushita, W. and Matsubayashi, Y. (2013) Identification of three hydroxypoline 5-O-arabinosyltransferases in *Arabidopsis thaliana*. Nat. Chem. Biol. 9, 726–730.
13) Ito, Y., Nakamoto, I., Motose, H., Iwamoto, K., Sawa, S., Dohmae, N. et al. (2006) Dodeca-CLE peptides as suppressors of plant stem cell differentiation. Science 313, 842–845.
14) Kondo, T., Sawa, S., Kinoshita, A., Mizuno, S., Kakimoto, T., Fukuda, H. et al. (2006) A plant peptide encoded by CLV3 identified by *in situ* MALDI-TOF MS analysis. Science 313, 845–848.
15) Shiu, S.H. and Bleecker, A.B. (2003) Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in Arabidopsis. Plant Physiol. 132, 530–543.

16) Ogawa, M., Shinozaka, H., Sakagami, Y. and Matsubayashi, Y. (2008) Arabidopsis CLV3 peptide directly binds CLV1 ectodomain. Science 319, 294.

17) Matsubayashi, Y., Takagi, L., Omura, N., Morita, A. and Sakagami, Y. (1999) The endogenous sulfated pentapeptide phytosulfokine-α stimulates tracheary element differentiation of isolated mesophyll cells of zinnia. Plant Physiol. 120, 1043–1048.

18) Fuluda, H. and Komamine, A. (1980) Establishment of an experimental system for the study of tracheary element differentiation from single cells isolated from the mesophyll of Zinnia elegans. Plant Physiol. 65, 57–60.

19) Kobayashi, T., Eun, C., Hanai, H., Matsubayashi, Y., Sakagami, Y. and Kamada, H. (1999) Phytosulphokine-α, a peptide plant growth factor, stimulates somatic embryogenesis in carrot. J. Exp. Bot. 50, 1123–1128.

20) Matsubayashi, Y., Takagi, L. and Sakagami, Y. (1997) Phytosulphokine-α, a sulfated pentapeptide, stimulates the proliferation of rice cells by means of specific high- and low-affinity binding sites. Proc. Natl. Acad. Sci. U.S.A. 94, 13357–13362.

21) Motose, H., Iwamoto, K., Endo, S., Demura, T., Sakagami, Y., Matsubayashi, Y. et al. (2009) Involvement of phytosulfokine in the attenuation of stress response during the transdifferentiation of zinnia mesophyll cells into tracheary elements. Plant Physiol. 150, 437–447.

22) Hanai, H., Matsuno, T., Yamamoto, M., Matsubayashi, Y., Kobayashi, Y., Kamada, H. et al. (2000) A secreted peptide growth factor, phytosulfokine, acting as a stimulatory factor of carrot somatic embryo formation. Plant Cell Physiol. 41, 27–32.

23) Igasaki, T., Akashi, N., Ujino-Ihara, T., Matsubayashi, Y., Sakagami, Y. and Shinozaka, K. (2005) Phytosulfokine stimulates somatic embryogenesis in Cryptomeria japonica. Plant Cell Physiol. 44, 1412–1416.

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

25) Yang, H., Matsubayashi, Y., Nakamura, K. and Sakagami, Y. (1999) Oryza sativa PSK gene encodes a precursor of phytosulfokine-α, a sulfated peptide growth factor found in plants. Proc. Natl. Acad. Sci. U.S.A. 96, 13560–13565.

26) Loivamaki, M., Stuhlwohldt, N., Deeken, R., Steffens, B., Roitsch, T. and Hedrich, R. et al. (2010) A role for PSK signaling in wounding and microbial interactions in Arabidopsis. Plant Physiol. 139, 348–357.

27) Sauter, M. (2015) Phytosulfokine peptide signalling. J. Exp. Bot. 66, 5161–5169.

28) Lorbiecke, R. and Sauter, M. (2002) Comparative analysis of PSK peptide growth factor precursor homologs. Plant Sci. 163, 321–332.

29) Matsubayashi, Y. and Sakagami, Y. (1999) Characterization of specific binding sites for a mitogenic sulfated peptide, phytosulfokine-α, in the plasma membrane fraction derived from Oryza sativa L. Eur. J. Biochem. 262, 666–671.

30) Matsubayashi, Y. and Sakagami, Y. (2000) 120- and 160-kDa receptors for endogenous mitogenic peptide, phytosulfokine-alpha, in rice plasma membranes. J. Biol. Chem. 275, 15520–15525.

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

32) Torii, K.U. (2004) Leucine-rich repeat receptor kinases in plants: structure, function, and signal transduction pathways. Int. Rev. Cytol. 234, 1–46.

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

34) Roberts, I., Smith, S., De Rybel, B., Van Den Broeke, J., Smet, W., De Cokere, S. et al. (2013) The CEP family in land plants: evolutionary analyses, expression studies, and role in Arabidopsis shoot development. J. Exp. Bot. 64, 5371–5381.

35) Delay, C., Imin, N. and Djordjevic, M.A. (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.

36) Drew, M.C. and Saker, L.R. (1975) Nutrient supply and growth of seminal root system in barley: II. Localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of root system. J. Exp. Bot. 26, 79–90.

37) Gansel, X., Munos, S., Tillard, P. and Gojon, A. (2001) Differential regulation of the NO3− and NH4+ transporter genes AtNrt2.1 and AtAmt1.1 in Arabidopsis: relation with long-distance and local controls by N status of the plant. Plant J. 26, 143–155.

38) Ruffel, S., Freixes, S., Balzerque, S., Tillard, P., Jeudy, C., Martin-Magniette, M.L. et al. (2008) Systemic signaling of the plant nitrogen status triggers specific transcriptome responses depending on the nitrogen source in Medicago truncatula. Plant Physiol. 146, 2030–2035.

39) Ruffel, S., Krouk, G., Risto, D., Shasha, D., Birnbaum, K.D. and Coruzzi, G.M. (2011) Nitrogen economics of root foraging; transitive closure of the nitrate-cytokinin relay and distinct systemic signaling for N supply vs. demand. Proc. Natl. Acad. Sci. U.S.A. 108, 18524–18529.

40) Ohkubo, Y., Tanaka, M., Tabata, R., Ogawa-Ohnishi, M. and Matsubayashi, Y. (2017) Shoot-to-root mobile polypeptides involved in systemic regulation of nitrogen acquisition. Nat. Plants 3, 17029.
41) Rouhier, N., Couturier, J. and Jacquot, J.P. (2006) Genome-wide analysis of plant glutaredoxin systems. J. Exp. Bot. 57, 1685–1696.

42) Okamoto, S., Tabata, R. and Matsubayashi, Y. (2016) Long-distance peptide signaling essential for nutrient homeostasis in plants. Curr. Opin. Plant Biol. 34, 35–40.

43) Zhou, W., Wei, L., Xu, J., Zhai, Q., Jiang, H., Chen, R. et al. (2010) Arabidopsis tyrosylprotein sulfotransferase acts in the auxin/PLETHORA pathway in regulating postembryonic maintenance of the root stem cell niche. Plant Cell 22, 3692–3709.

44) Fernandez, A., Drozdzocki, A., Hoogewijs, K., Nguyen, A., Beeckman, T., Madder, A. 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.

45) Meng, L., Buchanan, B.B., Feldman, L.J. and Luan, S. (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.

46) Whitford, R., Fernandez, A., Tejos, R., Perez, A.C., Kleine-Vehn, J., Vanneste, S. et al. (2012) GOLVEN secretory peptides regulate auxin carrier turnover during plant gravitropic responses. Dev. Cell 22, 678–685.

47) Casamitjana-Martinez, E., Hofhus, H.F., Xu, J., Liu, C.M., Heidstra, R. and Scheres, B. (2003) Root-specific CLE19 overexpression and the sol1/2 suppressors implicate a CLV-like pathway in the control of Arabidopsis root stem meristem maintenance. Curr. Biol. 13, 1435–1441.

48) Song, W., Liu, L., Wang, J., Wu, Z., Zhang, H., Tang, J. et al. (2016) Signature motif-guided identification of receptors for peptide hormones essential for root meristem growth. Cell Res. 26, 674–685.

49) Aida, M., Beis, D., Heidstra, R., Willemsen, V., Bilou, I., Galinha, C. et al. (2004) The PLETHORA genes mediate patterning of the Arabidopsis root stem cell niche. Cell 119, 109–120.

50) Galinha, C., Hofhus, H., Luijten, M., Willemsen, V., Bilou, I., Heidstra, R. et al. (2007) PLETHORA proteins as dose-dependent master regulators of Arabidopsis root development. Nature 449, 1053–1057.

51) Geldner, N. (2013) The endodermis. Annu. Rev. Plant Biol. 64, 531–558.

52) Pfister, A., Barberon, M., Alassimone, J., Kalmbach, L., Lee, Y., Vermeer, J.E. et al. (2014) A receptor-like kinase mutant with absent endodermal diffusion barrier displays selective nutrient homeostasis defects. eLife 3, e03115.

53) Doblas, V.G., Smakwoska-Luzan, E., Fujita, S., Alassimone, J., Barberon, M., Madalinski, M. et al. (2017) Root diffusion barrier control by a vasculature-derived peptide binding to the SGN3 receptor. Science 355, 280–284.

54) Robbins, N.E. 2nd, Trontin, C., Duan, L. and Dinneny, J.R. (2014) Beyond the barrier: communication in the root through the endodermis. Plant Physiol. 166, 551–559.

55) Matsubayashi, Y. (2011) Post-translational modifications in secreted hormone peptides in plants. Plant Cell Physiol. 52, 5–13.

56) Moore, K.L. (2003) The biology and enzymology of protein tyrosine O-sulfation. J. Biol. Chem. 278, 24243–24246.

57) Beisswanger, R., Corbeil, D., Vannier, C., Thiele, C., Dohrmann, U., Kellner, R. et al. (1998) Existence of distinct tyrosylprotein sulfotransferase genes: molecular characterization of tyrosylprotein sulfotransferase-2. Proc. Natl. Acad. Sci. U.S.A. 95, 11134–11139.

58) Ouyang, Y., Lane, W.S. and Moore, K.L. (1998) Tyrosylprotein sulfotransferase: purification and molecular cloning of an enzyme that catalyzes tyrosine O-sulfation, a common posttranslational modification of eukaryotic proteins. Proc. Natl. Acad. Sci. U.S.A. 95, 2896–2901.

59) Amano, Y., Tsubouchi, H., Shinohara, H., Ogawa, M. and Matsubayashi, Y. (2007) Tyrosine-sulfated glycopeptide involved in cellular proliferation and expansion in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. 104, 18333–18338.

60) Hanai, H., Nakayama, D., Yang, H., Matsubayashi, Y., Hirota, Y. and Sakagami, Y. (2000) Existence of a plant tyrosylprotein sulfotransferase: novel plant enzyme catalyzing tyrosine O-sulfation of preprophytosulfokine variants in vitro. FEBS Lett. 470, 97–101.

61) Wu, T., Kamiya, T., Umoto, H., Sotta, N., Katsushi, Y., Shigenobu, S. et al. (2015) An Arabidopsis thaliana copper-sensitive mutant suggests a role of phytosulfokine in ethylene production. J. Exp. Bot. 66, 3657–3667.

62) Kieliszewski, M.J. and Lampert, D.T. (1994) Extensin: repetitive motifs, functional sites, post-translational codes, and phylogeny. Plant J. 5, 157–172.

63) Pearce, C., Moura, D.S., Stratmann, J. and Ryan, C.A. (2001) Production of multiple plant hormones from a single polypeptide precursor. Nature 411, 817–820.

64) Ohyama, K., Shinohara, H., Ogawa-Ohnumi, M. and Matsubayashi, Y. (2009) A glycopeptide regulating stem cell fate in Arabidopsis thaliana. Nat. Chem. Biol. 5, 578–580.

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

66) Shinohara, H. and Matsubayashi, Y. (2013) Chemical synthesis of Arabidopsis CLV3 glycopeptide reveals the impact of hydroxyproline arabinosylation on peptide conformation and activity. Plant Cell Physiol. 54, 369–374.

67) Okamoto, S., Ohnumi, E., Sato, S., Takahashi, H., Nakazono, M., Tabata, S. et al. (2009) Nod factor/nitrate-induced CLE genes that drive HAR1-
mediated systemic regulation of nodulation. Plant Cell Physiol. 50, 67–77.

68) Okamoto, S., Shinohara, H., Mori, T., Matsubayashi, Y. and Kawaguchi, M. (2013) Root-derived CLE glycopeptides control nodulation by direct binding to HAR1 receptor kinase. Nat. Commun. 4, 2191.

69) Nikolovski, N., Rubtsov, D., Segura, M.P., Miles, G.P., Stevens, T.J., Dunkley, T.P. et al. (2012) Putative glycosyltransferases and other plant Golgi apparatus proteins are revealed by LOPIT proteomics. Plant Physiol. 160, 1037–1051.

70) Schnabel, E.L., Kassaw, T.K., Smith, L.S., Marsh, J.F., Olhroyd, G.E., Long, S.R. et al. (2011) The ROOT DETERMINED NODULATION1 gene regulates nodule number in roots of Medicago truncatula and defines a highly conserved, uncharacterized plant gene family. Plant Physiol. 157, 328–340.

71) Xu, C., Liberatore, K.L., MacAlister, C.A., Huang, Z., Chu, Y.H., Jiang, K. et al. (2015) A cascade of arabinosyltransferases controls shoot meristem size in tomato. Nat. Genet. 47, 784–792.

72) MacAlister, C.A., Ortiz-Ramírez, C., Becker, J.D., Feijo, J.A. and Lippman, Z.B. (2016) Hydroxyproline O-arabinosyltransferase mutants oppositely alter tip growth in Arabidopsis thaliana and Physcomitrella patens. Plant J. 85, 193–208.

73) Matsubayashi, Y. (2011) Small post-translationally modified Peptide signals in Arabidopsis. Arabidopsis Book 9, e0150.

74) Grebe, M. (2011) Unveiling the Casparian strip. Nature 473, 294–295.

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Profile

Yoshikatsu Matsubayashi was born in 1971 in Mie prefecture, Japan. He graduated from the School of Bio-Agricultural Sciences, Nagoya University in 1993 and received his Ph.D. degree from Nagoya University in 1997. He joined the Graduate School of Bio-Agricultural Sciences in Nagoya University as an Assistant Professor in 1999 and became Associate Professor in the same department in 2002. In 2011 he moved to National Institute for Basic Biology (NIBB) where he was appointed a Professor. He then moved to the Graduate School of Science, Nagoya University in 2014. He has been conducting research on peptide signaling in plants and identified various novel peptide hormones that are critical for plant growth, development, and environmental adaptation. For his accomplishments, he received the Japan Society for Bioscience, Biotechnology and Agrochemistry (JSBBA) Award for Young Scientists in 2001, Japanese Society of Plant Physiologists Young Investigator Award in 2008, Molecular Biology Society of Japan Mitsubishi Chemical Award in 2010, and the Japan Society for the Promotion of Science (JSPS) Prize in 2016.