Long-distance peptide signaling essential for nutrient homeostasis in plants

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Abstract

Organ-to-organ communication is indispensable for higher organisms to maintain homeostasis over their entire life. Recent findings have uncovered that plants, like animals, mediate organ-to-organ communication by long-distance signaling through the vascular system. In particular, xylem-mobile secreted peptides have attracted much attention as root-to-shoot long-distance signaling molecules in response to fluctuating environmental nutrient status. Several leguminous CLE peptides induced by rhizobial inoculation act as "satiety" signals in long-distance negative feedback of nodule formation. By contrast, *Arabidopsis* CEP family peptides induced by local nitrogen (N)-starvation behave as systemic "hunger" signals to promote compensatory N acquisition in other parts of the roots. Xylem sap peptidomics also implies the presence of still uncharacterized long-distance signaling peptides. This review highlights the current understanding of and new insights into the mechanisms and functions of root-to-shoot long-distance peptide signaling during environmental responses.

Introduction

Living organisms in nature that obtain nutrients from the environment often encounter a range of nutritional stresses due to fluctuations in nutrient availability. To maintain whole-body energy homeostasis under such conditions, nutritional response is controlled not only at the level of individual cells but also at the organismal level to orchestrate the systemic response of distant organs. Mammals transmit hunger and satiety signals from one organ to another by using long-distance mobile peptide hormones such as ghrelin and leptin [1,2]. Insects also produce peptide hormones to respond to nutritional and environmental demands [3].

These long-distance nutritional signaling pathways mediated by secreted peptides have long been considered to be conserved exclusively in animals that evolved advanced circulatory and endocrine systems. Recent studies, however, have demonstrated that plants also employ various long-distance mobile signals that mediate systemic response to transmit hunger and satiety signals through the vascular system. Particularly, "root-to-shoot-to-root" long-distance signaling pathways play a key role in response to environmental nutrient changes in plants [4-6]. These include CLAVATA3/ESR-related Root Signal (CLE-RS) and C-terminally Encoded Peptide (CEP) that function as root-to-shoot mobile signals mediating nitrogen (N) nutritional response [7,8]. These ascending signals are recognized in shoots by membrane-associated receptor kinases that eventually activate descending shoot-to-root secondary signals inducing systemic response of roots. Regulatory circuitry for environmental adaptation in plants may be much more dynamic than previously anticipated. This review focuses on the current knowledge of vascular-mediated long-distance peptide signaling, and discusses how root-to-shoot transport is driven at the whole-plant level.

CLE family peptides as a "satiety" signal during nodule formation

CLE peptides constitute one of the largest groups of secreted peptide signals in plants. Biochemical and physiological evidence indicates that 12- or 13-amino-acid peptides derived from the C-terminal regions of precursor polypeptides function as ligands regulating various stages of plant growth and development [9-12]. While CLE peptides have long been thought to act in local cell-to-cell communication within particular tissues, recent advances have shed light on their novel role as long-distance mobile signals required for systemic nutrient response.

In legumes, a subfamily of CLE peptides is involved in the long-distance regulation of nodulation. Leguminous plants establish symbiosis with soil bacteria, rhizobia, which convert atmospheric nitrogen into a form that is available for plant growth. Such a symbiotic

relationship is generally beneficial to both partners, but excess nodule formation is harmful for plants because of the high energy cost of nitrogen fixation. Host plants, therefore, strictly control the number of nodules in response to rhizobial inoculation or nitrogen status in soil through root-to-shoot-to-root long-distance "satiety" signaling [4,5]. This negative feedback loop consists of two long-distance signals, namely root-derived (root-to-shoot) and shoot-derived (shoot-to-root) signals.

In *Lotus japonicus*, CLE-RS1 and -RS2 peptides that are up-regulated in root stele and cortex by rhizobia inoculation or excess nitrate application act as root-derived ascending signals. CLE-RS peptides move shootward through the xylem and directly bind leucine-rich repeat receptor kinase (LRR-RK), HYPERNODULATION ABERRANT ROOT FORMATION 1 (HAR1), which activate descending shoot-to-root secondary signals that suppress excess nodulation [7,13-15] (Figure 1). CLE-RS peptides are 13-amino-acid peptides in which the 7th hydroxyproline residue is further modified with three L-arabinose sugars. This arabinose chain is critical for the biological activity and receptor binding activity of CLE-RS peptides. In addition, glycosylation generally provides increased resistance to proteolytic degradation. Rhizobia-inducible CLE peptides and HAR1 orthologues have also been identified in other legume species [16-21], indicating that CLE-mediated long-distance signaling is conserved in leguminous plants.

HAR1 promoter activity is detected in phloem tissues [22]. This spatial localization leads to the notion that the shoot-derived secondary signal, which is induced downstream of HAR1, is sent back to the roots via phloem. A currently proposed candidate for this shoot-derived signal is cytokinin (CK), whose biosynthesis is upregulated in shoots by CLE-RS overexpression or rhizobial infection [23]. Shoot application of cytokinin suppresses root nodule formation even in *har1* mutants. Collectively, these findings suggest that roots communicate with each other by means of root-to-shoot-to-root long-distance signaling.

CEP family peptides as "hunger" signals upon nitrogen starvation

Nitrogen is one of the most essential macronutrients for plant growth and development. Plants uptake N from soil mainly in the form of nitrate; however, nitrate is often distributed heterogeneously in soil. Therefore, plants have systemic long-distance signaling by which N-starvation on one side of the root leads to a compensatory N uptake on the other, N-rich side [6,24].

The *Arabidopsis* C-terminally Encoded Peptide (CEP) family is another class of long-distance signaling molecule that transmits the signal triggered by N-starvation from root to shoot. CEP family peptides are 15-amino-acid residues long and are generated by

posttranslational proline hydroxylation and proteolytic processing [8,25]. The *Arabidopsis* genome contains 15 CEP genes [26,27], among which 7 are upregulated approximately 10-fold in response to N-starvation [8]. They are specifically expressed in the stele of lateral roots and are loaded into xylem vessels to be transported to the shoot. CEP family peptides are then detected by the receptor kinase CEP Receptor 1 (CEPR1), expressed in the vascular tissues of leaves, which induces the production of a putative shoot-derived secondary signal that upregulates nitrate transporter genes such as *NRT2.1* in distant parts of roots to compensate for local N-starvation (Figure 1). Because both CEP family peptides and CEPR1 are widespread among seed plants that have true branching roots [26,27], the CEP-CEPR signaling module appears to be evolutionarily conserved in all seed plants.

In mammals, the 28-residue "hunger" hormone ghrelin is produced by stomach cells under starvation conditions and is secreted into circulation [1]. Ghrelin acts as an endogenous ligand for the growth hormone secretagogue (GHS) receptor [1] and stimulates the synthesis and secretion of several neuropeptides in the hypothalamus that regulate feeding [28]. In this context, plants have evolved long-distance signaling pathways in a manner analogous to mammals, to convert a "hunger" signal into a "nutrient intake-facilitating" signal. CEP family peptides can thus be regarded as a plant version of the hunger signal.

In *Medicago truncatula*, overexpression of *MtCEP1* promotes nodule formation [29]. Conversely, a loss-of-function mutant of the *M. truncatula CEPR1* ortholog *COMPACT ROOT ARCHITECTURE 2* (*CRA2*) forms a decreased number of nodules, which is opposite to that seen in *MtCEP1*-overexpressing plants [30]. Grafting experiments suggest that CRA2 regulates nodule formation systemically but controls lateral root development locally [30], leaving the possibility that CEP family peptides also act as local signals regulating root system architecture [31,32]. These observations further support the concept that the CEP family functions as a hunger signal, promoting nutrient intake.

Other candidates for long-distance signaling peptides

Among the numerous CLE family peptides in plant genomes, the question remains whether only nodulation-related CLE peptides are involved in long-distance signaling. Although further studies will be needed, two lines of evidence suggest that several more CLE peptides mediate root-to-shoot long-distance communication. First, grafting experiments in *Arabidopsis* have shown that *CLE6*-overexpressing rootstock partially restores flowering stem elongation in a gibberellic acid (GA)-deficient mutant [33]. CLE6 is expressed in roots, especially in stele cells, at branch points and is upregulated by GA treatment, suggesting that root-expressed CLE6 may be involved in shoot growth under GA action in *Arabidopsis*.

Second, a soybean CLE family peptide, Xylem sap-associated peptide 4 (XAP4)/*Gm*CLE32, was identified in xylem sap by exhaustive peptidomics analysis [34]. XAP4/*Gm*CLE32 is specifically expressed in the stele of roots and is strongly induced by flooding stress, suggesting its involvement in environmental response.

Other candidates for long-distance signaling include tyrosine-sulfated peptides, named XAP1, 2, 3, and 7, that have been identified by xylem sap peptidomics in soybean. XAP1 and XAP3 are expressed in root stele and have been shown to translocate from the roots to shoots when they are expressed in hairy roots with their own promoter. It has been demonstrated that tyrosine-sulfation is catalyzed by tyrosylprotein sulfotransferase (TPST) and that mutations in *TPST* exhibit pleiotropic phenotypes such as short roots, small leaves, and shorter inflorescence [35]. Therefore, it is tempting to speculate that these sulfated peptides are involved in certain stages of plant development by mediating root-to-shoot communication.

The Casparian strip may facilitate xylem loading of peptides in roots

All of the peptides identified in xylem sap are expressed inside the endodermis [8,34,36] in which the walls are covered with a band of suberin and lignin called the Casparian strip [37]. The Casparian strip blocks apoplastic water movement between the cortex and stele and helps prevent the direct influx of salts and the invasion of pathogens into the stele. Loss of functional Casparian strips has been shown to lead to increased leakage of solutes back out of the stele [38]. Thus, preferential expression of xylem-mobile peptides inside the endodermis might be because the Casparian strip facilitates long distance signaling by preventing diffusive loss of peptides (Figure 2). Peptides secreted by the stele cells are loaded into the xylem probably by passive diffusion rather than a transporter-mediated pathway, and are then transported from roots to shoots by the xylem stream.

Driving force for root-to-shoot long-distance peptide signaling

In animals, the heart pumps blood through the circulatory system by which long-distance signaling molecules are transported from one organ to another. In plants, transpiration flow and root pressure generate a positive pressure that forces sap up the xylem towards the leaves. Although varying somewhat among species and conditions, the velocity of the xylem stream is estimated to be 1 to 5 mm/s in crops [39] which is fast enough to transport signal molecules from roots to shoots in response to environmental stimuli.

In long-distance signaling, the dilution of signals by excess body fluids can often be problematic for transmitting precise information to distant organs and/or tissues. Indeed, the xylem is filled with water that is absorbed through the roots. In this situation it may be

unavoidable that signal molecules are diluted by excess water in the xylem. Based on ion peak intensities of LC-MS analyses, concentrations of native CLE-RS2 and CEPs in xylem sap were estimated to be between 10 pM and 100 pM. These concentrations are somewhat lower than those required for binding to receptors with binding constants typically in the nanomolar range [11,40-42]. Long-distance mobile peptides, therefore, must be concentrated to a high enough level for receptor activation.

Receptors for CLE-RS and CEP family peptides are expressed in leaf vascular tissues [8,22], where the solute concentration in xylem sap is drastically increased by stomatal transpiration. In addition, leaf vascular tissues are a favorable place to send back secondary signals to the whole plant through the phloem. It is therefore plausible that plants may employ transpiration to amplify the diluted long-distance signals from the roots, and that may be the reason why plants utilize the root-to-shoot-to-root pathway for root-to-root communication (Figure 2).

Concluding remarks

In plants, secreted peptides have long been thought to exclusively mediate short-distance cell-to-cell signaling involved in growth and development. Recent studies, however, have provided a novel functional model in which peptides respond to environmental stimuli and mediate long-distance communication, especially from the roots to the shoots. In addition, plant genomes encode numerous secretory peptides whose functions have yet to be explored. 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 currently assumed. We predict that the observed cases of long-distance signaling peptides represent only the tip of the iceberg, and more examples are likely to be found in the near future.

Figure legends

Figure 1

Models for long-distance signaling mediated by secreted peptides. CLE-RS1/2 peptides are induced by rhizobial inoculation, and CLE-RS2 also responds to nitrate. CLE-RS1/2 peptides transmit to the shoots to signal that sufficient nitrogen is available to roots. HAR1 receptor recognizes the CLE-RS peptides and sends CK to roots to suppress further nodule formation. CEPs are induced by N-starvation and transmit to the shoots that roots are hungry for nitrogen. Recognition of CEPs by CEPR1 leads to the generation of a putative shoot-to-root signal that promotes N-uptake in distant parts of the roots to compensate for local N-starvation.

Figure 2

A possible model for the translocation of long-distance signaling peptides. The peptides are expressed in the inner region of endodermis covered with the Casparian strip that blocks apoplastic water movement between the cortex and stele. Peptides expressed in the stele are loaded into the xylem probably by passive diffusion and are then transported to shoots by the xylem stream together with excess water. In leaves, the peptides are concentrated by transpiration, bind to specific receptors, and drive downstream signals.

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(a)

HAR1

CK?

Xylem

Phloem

CLE-RS
("satiety" signal)

N-acquisition

Inhibition of
nodule formation

Nodule formation

Nitrate

N-rich

(b)

CEPR

Secondary signal

Xylem

Phloem

CEP
("hunger" signal)

OH

N-acquisition

NRT2.1

Promotion of
N-uptake

Nitrate

N-starved

Transpiration

Casparian strip

Xylem stream

H_2O

● Peptide signal