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Sonali Roy
Tennessee State University

Lena Maria Müller
University of Miami

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Feature Review

A rulebook for peptide control of legume–microbe endosymbioses

Sonali Roy [1,*] and Lena Maria Müller [2,*]

Plants engage in mutually beneficial relationships with microbes, such as arbuscular mycorrhizal fungi or nitrogen-fixing rhizobia, for optimized nutrient acquisition. In return, the microbial symbionts receive photosynthetic carbon from the plant. Both symbioses are regulated by the plant nutrient status, indicating the existence of signaling pathways that allow the host to fine-tune its interactions with the beneficial microbes depending on its nutrient requirements. Peptide hormones coordinate a plethora of developmental and physiological processes and, recently, various peptide families have gained special attention as systemic and local regulators of plant–microbe interactions and nutrient homeostasis. In this review, we identify five 'rules' or guiding principles that govern peptide function during symbiotic plant–microbe interactions, and highlight possible points of integration with nutrient acquisition pathways.

Highlights

Plant interactions with arbuscular mycorrhizal fungi, beneficial soil bacteria, and nutrient homeostasis are optimized by an interconnected network of peptide signals.

Symbiosis-regulating signaling peptides are members of large protein families, often with a variety of functions in plant physiology and development.

The mechanism of peptide-signaling specificity in the context of plant–microbe interactions, nutrient homeostasis, and cross-kingdom peptide mimicry involves antagonism and coordination between individual peptide signals.

Although many of the symbiosis-associated peptide signaling pathways converge at common downstream signaling hubs and intersect with phytohormone signaling, the signaling outcomes are, at least partially, unique.

Plants interact with microbes to optimize nutrient acquisition

Plants engage in mutually beneficial relationships with microbes to optimize their nutrient uptake (Box 1). Arbuscular mycorrhiza (AM) **symbiosis** (see Glossary) is an interaction occurring between almost 70% of all land plants and fungal endosymbionts of the subphylum Glomeromycotina, which provide the plant with mineral nutrients (e.g., phosphorus, P) and other benefits (e.g., increased resistance to biotic and abiotic stresses) [1]. Similarly, a limited number of plant groups, including legumes, can engage in root nodule (RN) symbiosis with nitrogen (N)-fixing bacteria, such as rhizobia [2]. In exchange for nutrients, the host provides AM fungi or N-fixing bacteria with photosynthetically fixed carbon. The plant host initiates an interaction with symbiotic microbes to meet its nutrient demands, which requires extensive signaling at the cellular, tissue, and systemic levels to accommodate the symbiont. **Peptide hormones** are short-chain polypeptides, ranging from five to 60 residues, that can act as regulators of symbiosis establishment when perceived by cell surface receptors [2,3] (Box 2). Numerous discoveries in recent years resulted in an enormous expansion of our understanding of peptide function during AM or RN signaling and its integration with P and N homeostasis, respectively. Here, we synthesize five emerging principles that govern the interconnected, peptide-mediated signaling pathways regulating plant–microbe symbioses and nutrient acquisition.

One: concerted action of multiple peptide signals fine-tunes plant nutrient homeostasis and symbioses with microorganisms

Given that N and P are essential for the formation of biological molecules, such as amino acids and nucleotides, their acquisition is a tightly regulated process that involves both positive and negative regulators of nutrient homeostasis [4]. Plants can directly take up N and P from the soil, but, in legumes, the ability to associate with rhizobia and AM fungi adds an additional layer of complexity. How do legumes distinguish and prioritize between different mechanisms of nutrient acquisition and commit to any one for optimal growth in a marginal environment? The coordination of symbiosis and N and P foraging by roots is orchestrated by a multitude of interconnected peptide

[1]College of Agriculture, Tennessee State University, Nashville, TN 37209, USA
[2]Department of Biology, University of Miami, Coral Gables, FL 33146, USA

*Correspondence:
sroy3@tnstate.edu (S. Roy) and
lena.mueller@miami.edu (L.M. Müller).

Box 1. Plant symbioses with beneficial microbes

To optimize their nutrient uptake, many land plants engage in mutually beneficial interactions with soil microbes. While ~70% of all terrestrial plant species interact with Glomeromycotina fungi to engage in AM symbiosis, root nodule (RN) symbiosis between plants and beneficial soil bacteria is evolutionarily younger and restricted to four plant orders (Fabales, Fagales, Cucurbitales, and Rosales) [128]. Since both AM and RN symbiosis depend on the microbe residing inside host tissue, these relationships are referred to as 'endosymbiosis'. During RN symbiosis, rhizobia are harbored in specialized root organs (nodules), where they 'fix' atmospheric nitrogen (N_2) to plant-usable ammonia in an energy-requiring reaction catalyzed by nitrogenase for their plant host in exchange for carbon derived from photosynthesis [2]. By contrast, no specialized organs are required for AM symbiosis. Instead, AM fungi invade the root cortex cells, where they form intricately branched hyphal structures (arbuscules) that function in bidirectional nutrient exchange. In exchange for carbon provided by the host plant, AM fungi supply the plant with various mineral nutrients [predominantly phosphorus (P), but also N, potassium, sulfur, and zinc] taken up from the soil via their vast extraradical hyphal network [129].

Both RN and AM symbiosis are heavily regulated by nutrients and suppressed when plants can meet their nutrient demands without symbiotic microbes; for example, in the presence of a high exogenous N supply, RN symbiosis is inhibited [130]. Similarly, AM symbiosis is strongly suppressed by exogenous P supply [131], but other nutrients, including N, also have a role in its regulation [31,132,133]. AM and RN symbiosis initiation is regulated by a crosstalk between hosts and microbes. Development of both symbioses depends on an early dialog between the host and microbes followed by activation of the 'common symbiosis' signaling pathway [134], which comprises a shared set of core genes required for the reprogramming of the host cells before the accommodation of symbiotic microbes [134]. In addition, both symbioses are governed by mechanistically similar, systemic autoregulation pathways, which restrict the formation of additional nodules or AM fungal colonization once a critical symbiosis level is reached [66]. These negative feedback loops are thought to prevent oversequestration of carbon by the microbial symbiont.

signaling networks downstream of rhizobia-secreted Nod-factors or the elusive Myc-factors (Box 3). Such peptide signaling networks include root-to-shoot 'N-hunger' signals, such as the C-terminally encoded peptides (CEPs), which mediate enhanced uptake of N in N-poor soils and stimulate nodulation [5–7]. By contrast, members of another peptide family, the CLAVATA3/ESR (CLE) peptides, limit the number of nodules that form on legume roots, possibly to balance carbon expenditure and N acquisition through symbiotic N fixation [8]; recent research revealed that AM symbiosis and P acquisition are also regulated by similar signaling mechanisms (see below) [9,10]. Through use of loss-of-function mutants and gain-of-function approaches (Box 4),

Box 2. Peptide hormone characteristics and perception

Peptide hormones, defined as mobile, proteinaceous signaling molecules of 5–60 amino acids, often derived from longer polypeptides called pre-propeptides, are encoded within the plant genome [20,106,135]. These can serve as cross-kingdom signals between hosts and their microbial symbionts (see Box 3 in the main text) or act as signals within the host plant itself to mediate cell–cell signaling between neighboring cells or systemically facilitate organ–organ signaling by traveling through the vascular tissue.

The function of peptide hormones depends on their perception by cognate receptor-like kinases (RLKs), which selectively bind peptide ligands with their extracellular domains [136]. So far, most peptides, including CLAVATA3/ESR (CLE), C-terminally encoded peptides (CEPs), RGF, and PSK, have been found to interact with leucine-rich repeat RLKs (LRR-RLKs); however, other peptides, such as RALF, interact with RLKs of the *Catharanthus roseus* RLK1-like (*Cr*RLK1L) subfamily [137]. In either case, the intracellular serine/threonine kinase domain of the RLK is required for signal transduction upon ligand binding. In plants, most peptide receptors are found in LRR-RLK clades X, XI, and XIII [138]. For example, legume orthologs of the arabidopsis (*Arabidopsis thaliana*) clade XI LRR-RLK CLAVATA1 (*At*CLV1) include *Mt*SUNN, *Lj*HAR1, and *Gm*NARK. In arabidopsis, CLV1-type LRR-RLKs typically act in higher order complexes as homo- or heterodimers, or in association with CLV2-type receptor proteins and CRN-type membrane kinases [60], orthologs of which are also implicated in CLE perception during symbiosis [64–66,139]. In *Medicago truncatula*, clade XI contains 100 proteins, ~70% of which are transcriptionally regulated upon infection by rhizobia or AM fungi (Figure I) [151]. Interestingly, when comparing clade XI of *M. truncatula* with that of the nonmycorrhizal and nonnodulating arabidopsis, it becomes apparent that the clade is massively expanded in the legume (Figure I), and that most of these genes have no described function. While the *M. truncatula* lineage experienced massive gene duplication in general [140], the increased number of receptors may also reflect an increased need for signaling pathways to allow the legume to deal with signals associated with different symbionts, as indicated by their heightened expression during RN and AM symbiosis (Figure I).

Glossary

Autoregulation of mycorrhizal symbiosis (AOM): negative regulatory process within some land plants that restricts the percentage of roots colonized by AM fungi.
Autoregulation of nodulation (AON): negative regulatory process within legumes that limits the number of nodules formed on their roots.
Common symbiosis signaling pathway (CSSP): core signaling pathway components shared downstream of both AM and RN symbiont perception but upstream of the distinct developmental response specific to accommodation of the fungal or bacterial partner.
Cross-kingdom signaling: communication between two or more organisms across taxonomic groups called Kingdoms (Animalia, Plantae, Fungi, Protista, Archaea/Archaebacteria, and Bacteria/Eubacteria); also known as interkingdom signaling.
Leucine-rich repeat receptor-like kinases (LRR-RLKs): a family of protein kinases with a leucine-rich repeat extracellular domain, a single transmembrane domain, and an intracellular kinase domain. The LRR-RLK family in plants can be further subdivided into 19 subfamilies (clades I–XIII, VI-1, VI-2; VII-1, VII-2, XIII-1, and XIII-2) based on their amino acid sequence similarity.
Peptide hormones: small signaling peptides that display characteristics of plant hormones, such as non-cell-autonomous activity, perception by cell surface receptors, and control of physiological traits by regulation at the molecular level. Peptide hormones differ from classical hormones, which are typically end products of metabolic pathways, in terms of how they are biosynthesized.
Small signaling peptides (SSPs): a class of regulatory molecules derived from a larger polypeptide and encoded within the genome of an organism. SSPs may act as signals in their role as peptide hormones, as antimicrobial peptides, or as cell-penetrating peptides. All peptide hormones are SSPs but not all SSPs are peptide hormones.
Symbiosis: a close and prolonged relationship between two organisms. For simplicity, within the context of this article, 'symbiosis' relates exclusively to a mutually beneficial relationship

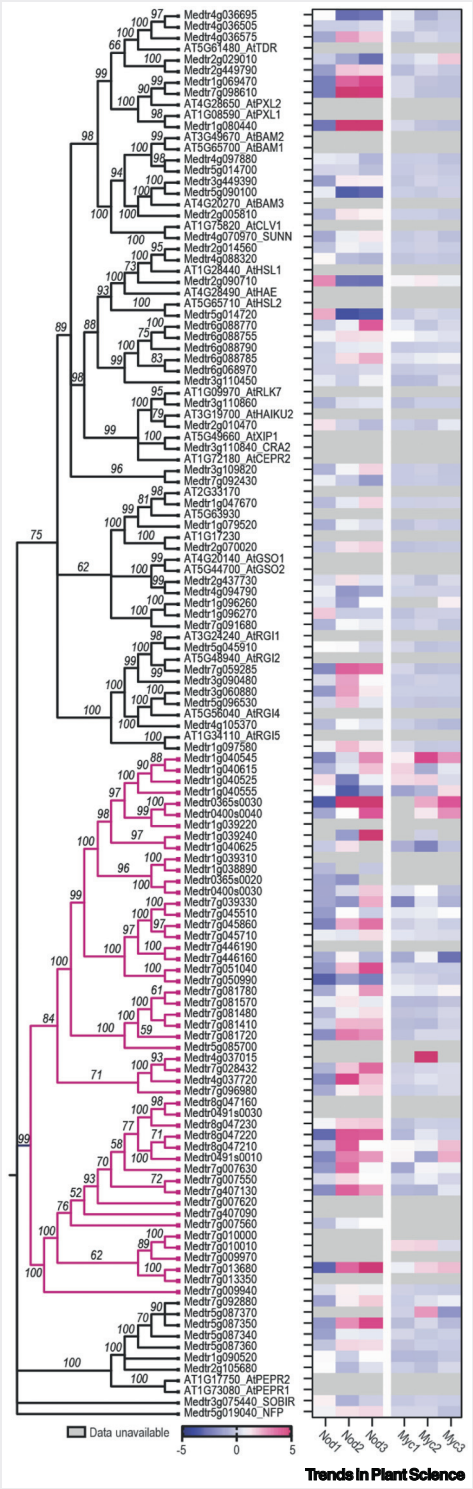


Figure I. Maximum likelihood phylogenetic tree depicting interrelationships between Clade XI leucine-rich receptor-like kinases (LRR-RLKs) encoded within arabidopsis (*Arabidopsis thaliana*) and *Medicago truncatula* genomes. The LysM receptor-like kinase nod factor perception (NFP) was used as an outgroup. The corresponding heat map shows a log twofold change in expression of the genes under symbiotic conditions upon infection with the rhizobium *Sinorhizobium meliloti* or the arbuscular mycorrhizal (AM) fungus *Rhizophagus irregularis*. Gene expression data were obtained from the publicly available database MtSSPdb [149]. Tree branches in magenta highlight the phylogenetic clade without arabidopsis orthologs. Tree was constructed using Mega X with 1000 bootstrap iterations and modified for clarity using Adobe Illustrator. Abbreviations: Myc1, infected roots 8 dpi; Myc2, infected roots 13 dpi; Myc3, infected roots 27 dpi; Nod1, Nodule bumps 4 dpi; Nod2, Nodules 14 dpi; Nod3, Nodules 28 dpi.

between two partners both of which derive nutritional benefits from their association. A symbiotic relationship in which one symbiont lives inside the other is called ‘endosymbiosis’.

Box 3. Peptides can act as cross-kingdom signals between legumes and their microbial partners

Mutually beneficial relationships are based on effective communication between both partners. In fact, the entire process is triggered when microbes, such as AM fungi or rhizobia, perceive plant-produced strigolactones (SLs) or flavonoids and, in turn, produce their own lipochitooligosacccharide (LCO) signals called Myc- or Nod- factors, respectively. Microbial symbionts also produce effector molecules, which are proteins or metabolites expressed by plant-associated microbes that enhance colonization of the host. Although best studied in plant pathogens, many symbionts produce effectors, including small, secreted peptides, to boost their infectivity [141,142]. For example, the AM fungus *Rhizophagus irregularis* produces **small signaling peptides (SSPs)** upon perception of SL. The SL-induced putative secreted protein, *Ri*SIS1, was shown to positively regulate root colonization [143]. Mycorrhizal signaling peptides are likely post-translationally modified and/or cleaved into smaller peptides, but their mechanistic functions remain unknown [124].

Several plant peptides can also act as direct signals to the microbial symbiont. The genome of the model legume *Medicago truncatula* has almost 800 genes that encode nodule-cysteine-rich (NCR) peptides, the length of which varies from 24 to 65 amino acids [20]. NCR peptides have antimicrobial activity and bring about 'terminal differentiation' of bacteroides within the nodules required for functional N fixation [144]. Terminal differentiation of rhizobia precedes N fixation and refers to a process during which rhizobia cease to divide, become swollen, undergo endoreduplication, and become nonmotile [92,98,145]. NCR peptides can also mediate symbiotic scrutiny, as evidenced by two genes encoding *NITROGEN FIXATION SPECIFICITY 1* and *2* (*NFS1* and *NFS2*), which are involved in actively terminating bacterial interactions that form inefficient symbiosis with the host [146,147]. This allows the plant to selectively host only rhizobial strains with high N_2-fixation capacity.

NCR peptides share high homology with another family of cysteine-rich peptides called defensins. During AM symbiosis, the defensin-like peptide Mt*DefMd1* accumulates in colonized cortex cells during late stages of the arbuscule lifecycle [148]. Based on high homology with NCRs, Mt*DefMd1* was proposed to have a role in the control of arbuscule lifespan and/or arbuscule degeneration [148]. The large size of the defensin and NCR gene families and possible functional redundancies among family members have so far complicated functional experiments, but future research will elucidate the role of these key peptide families and others involved in microbial partner selection.

researchers found that an optimal concentration is required for the proper function of an individual peptide (Figure 1A) [8,11]. These findings support a model involving partially antagonistic or additive peptide functions, which are required to balance nutrient homeostasis and symbiosis over time

Box 4. Tools for studying peptide hormones in plants

Tools available to researchers interested in investigating peptide hormones include databases to help determine whether a short ORF encodes a peptide. The SSP prediction tool available from https://MtSSPdb.zhaolab.org [149] uses four criteria to designate a peptide: (i) its length should be less than 250 amino acids; (ii) presence of N-terminal secretion signal peptide cleavage sites; (iii) homology with previously identified plant signaling peptide families as a criterion to classify putative peptides into three groups (known peptides, likely known, and putative peptides); and (iv) the absence of any transmembrane domains, since secreted peptides are unlikely to be membrane bound.

Tools also include databases to identify orthologs of peptides in different plant species: Using the BLAST feature available from http://bioinformatics.psb.ugent.be/webtools/PlantSSP/ [150], users are provided with a list of putative peptide orthologs in different plant species, including maize, rice, and poplar.

Both databases provide a comprehensive overview of peptide families in plants and users are recommended to explore the two websites for additional tools, such as gene expression and effects of synthetic peptide on different physiological parameters.

Experimental tools are also available to identify peptides *in planta* including the use of mass spectrometry to elucidate the sequence of biologically active peptides and their post-translational modifications *in vivo* [17]. Gain-of-function experiments, by application of chemically synthesized peptides, are a fast and convenient way to investigate peptide function. Researchers can outsource peptide synthesis to companies such as Pepscan, Biomatik, Genscript, or Millipore Sigma (among others) by providing the amino acid sequence (e.g,. *At*CEP1 DFRPTNPGNSPGVGH), any requisite post-translational modification, if feasible (e.g., tyrosine sulfation or proline hydroxylation) and quantity required (e.g., >1 mg). Activity of the peptides can be tested by adding them to growth media or directly to the experimental organisms at nanomolar–micromolar concentrations. For each experimental system, testing of multiple peptide concentrations should be considered to differentiate pleiotropic from meaningful effects. Experimental tools are also available to study the effects of peptide loss of function, including artificial mRNA technology, antisense RNA knockdown, and insertional mutagenesis [8,10,14]. Over the next decade, the use of genome editing with clustered regularly interspaced short palindromic sequences (CRISPR/Cas9) will likely have a major role in understanding gene function in multigenic peptide families with redundant or compensatory gene expression.

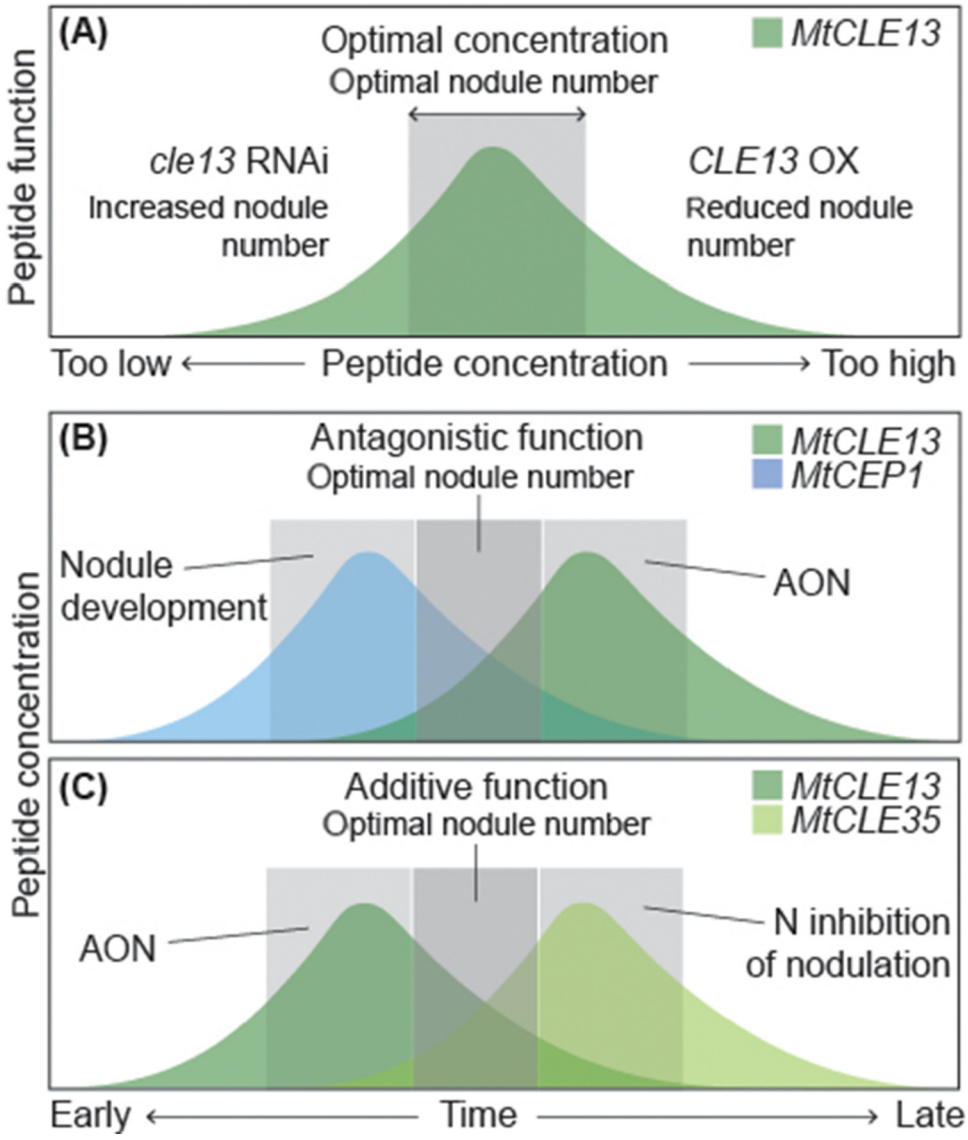


Figure 1. Model proposing that peptide function depends on concentration and timing. (A) An optimal concentration of peptide (e.g., *MtCLE13*) is required for fine-tuning nodule numbers. Reduced expression of *MtCLE13*, such as by RNAi, results in increased nodule numbers [11], whereas overexpression (OX) of *MtCLE13* results in reduced nodule numbers [8]. (B) Nitrogen (N) starvation induces the expression of *MtCEP1*, which promotes root susceptibility for rhizobial infection and nodule development [5]. *MtCLE13* is induced from early nodule development onward and negatively regulates nodule number after a certain concentration threshold is reached (autoregulation of nodulation, AON) [8]. We hypothesize that a certain combined concentration of these antagonistically acting peptides is required for optimal nodule number. (C) *MtCLE13* is induced by rhizobia and negatively regulates nodule number (AON). *MtCLE35* is induced by rhizobia and high N availability [12,13,15]. We hypothesize that the combined function of both rhizobia-induced peptides represses nodulation and that *MtCLE35* continues to repress nodulation for as long as sufficient N is available to the plant.

(Figure 1B,C) [5,12–15]. As detailed in the following paragraphs, the staggered timing of peptide induction and/or their relative concentration, rather than the presence or absence of a single peptide, likely allows the plant to dynamically respond to ever-changing environmental conditions.

CEP and CLE peptides fine-tune plant symbioses systemically

CEPs are 15-amino acid-long, post-translationally modified peptides, which are produced in the root and loaded into the xylem for long-distance transport to the shoot [16,17]. Low N-induced *Medicago truncatula CEP1*, *MtCEP2*, and *MtCEP12*, as well as Nod-factor induced *MtCEP7*, act as positive regulators of nodulation and stimulate rhizobial infection when N is limited [5,14,18,19]. Ectopic overexpression of *MtCEP1* or application of the synthetic peptide *Mt*CEP1 enhances nodule number [5]. Conversely, downregulation of another member of the CEP family, *MtCEP7,* by RNAi, resulted in reduced nodule numbers, indicating that *Mt*CEP7 acts as a positive regulator of nodulation by maintaining root competence for nodule formation after initial infection by rhizobia [14,18].

By contrast, CLE peptides have been established as negative regulators of RN and AM symbiosis. Several genes encoding CLE peptides were found to be induced in roots in response to plant interactions with both AM fungi and rhizobia, as well as with macronutrients, such as N and P [9,10,20–24]. Fully processed CLE peptides are 12–13-amino acids long and often post-translationally modified by proline hydroxylation and arabinosylation [25]. Functional characterization in legumes revealed that RN-induced CLE peptides (*Mt*CLE12, *Mt*CLE13, *Mt*CLE35; *Lotus japonicus* CLE-RS1, *Lj*CLE-RS2, *Lj*CLE-RS3; *Phaseolus vulgaris* RIC1, *Pv*RIC2; and *Glycine max* RIC1, *Gm*RIC2) act as systemic, negative regulators of nodule number in a signaling pathway referred to as '**autoregulation of nodulation**' **(AON)** (for a recent review, see [26]). A similar autoregulatory pathway also fine-tunes plant root colonization by AM fungi (**autoregulation of mycorrhizal symbiosis**; **AOM**). In *M. truncatula*, AOM is mediated by AM-induced *MtCLE53*, which, when overexpressed, negatively regulates fungal root colonization [9,10].

Expression of several CEP and CLE peptides is regulated by plant nutrient status

As described earlier, *MtCEP1*, *MtCEP2*, and *MtCEP12* are induced in roots grown under low N conditions and promote nodule formation [5,14,18,19]. By contrast, some RN-induced *CLE* genes that negatively regulate nodule numbers (e.g., *MtCLE35*, *LjCLE-RS2*, and *LjCLE-RS3*) are also induced by high N [13,15,27,28]. In addition, N- but not RN-, induced CLE genes were identified, which also negatively affect nodule numbers (e.g., *LjCLE40*, *PvNIC1*, and *GmNIC1*) [27,29,30]. The antagonistic function of CEPs and CLEs in symbiosis control supports the hypothesis that CEPs initially establish root competency for nodulation based on N availability. After a certain nodule number is reached and/or sufficient N is available, *CLE* expression increases and limits further nodulation to conserve carbon (Figure 1B,C). Likewise, several *CLE* genes have been described to be induced by high P and AM, or high P alone [9,10,22], and functional studies indicate that the P-induced *MtCLE33* negatively regulates AM fungal root colonization in *M. truncatula* [9], indicating that CLE peptides contribute to the integration of plant P status and AM symbiosis. Interestingly, while there appears to be a partial overlap of RN- and N-induced and of AM- and P-induced CLE peptides, no CLE has been described as being induced by both symbioses. This indicates that the conditional expression of *CLE* genes is specific, and the observation that some CLEs are induced by RN and N or by AM and P may be due to increased nutrient availability via the symbiotic microbes. Given that AM symbiosis is also partially regulated by N [31], it will be interesting to functionally determine whether N-induced CLEs are involved in N regulation of AM. While there are no reports to date that suggest a role for CEPs during AM symbiosis, it is conceivable that analogous regulatory processes govern plant interactions with beneficial fungi.

Most CLEs described so far are expressed in line with a function as 'satiety' signals (i.e., sufficient nutrients and/or symbiont presence). However, recent evidence suggests that RN-induced *Pv*RIC1 and *Pv*RIC2 are also induced in roots grown under low P conditions in the absence of symbiosis [32]. While this may be one mechanistic explanation for the well-known phenomenon

that RN symbiosis is inhibited in low P conditions [33], it raises the question of how symbiont- or nutrient-specific induction of CLE signals is regulated. Interestingly, the transcription factor NODULE INCEPTION (NIN), a RWP-RK-containing transcription factor originally identified as a critical regulator of nodule organogenesis [34], appears to have a central role in the induction of various root-derived peptide signals: it induces the induction of the positive regulator of nodule number *MtCEP7*, as well as of the negative regulators *MtCLE13, MtCLE35,* and *LjCLE-RS1/-RS2* [14,28,35]. It is likely that additional transcriptional regulators are required to specify peptide induction in response to distinct environmental cues. In line with this hypothesis, NIN was only required for *MtCLE35* induction in response to rhizobia, whereas *MtCLE35* induction in response to high N was dependent on the transcription factor NLP1 [28].

Closely related peptide hormones differ in their spatiotemporal expression

Genes encoding peptide hormones differ not only in their regulation by distinct nutrient or symbiosis cues, but also in their spatiotemporal expression. For example, AM-induced *MtCLE53* is expressed in the vascular tissue near colonized cortex cells [9], whereas RN-induced *MtCLE12* and *MtCLE13* are expressed in nodule meristems [8]. *MtCEP1* expression was detected in the vascular tissue, root tips, and young lateral roots, while *MtCEP7* expression was induced in the root epidermis following rhizobial inoculation [5,14]. In addition, the precise timing of induction appears to differ between closely related RN-induced *MtCLE* genes [8,13,15] and, although the functional relevance of such a staggered induction has not yet been investigated, it may contribute to signaling outcomes (Figure 1B,C). The precise timing and spatial regulation of peptide expression, based on factors such as changing nutrient availability or symbiont interactions, likely allows the plant to dynamically adapt to a changing environment.

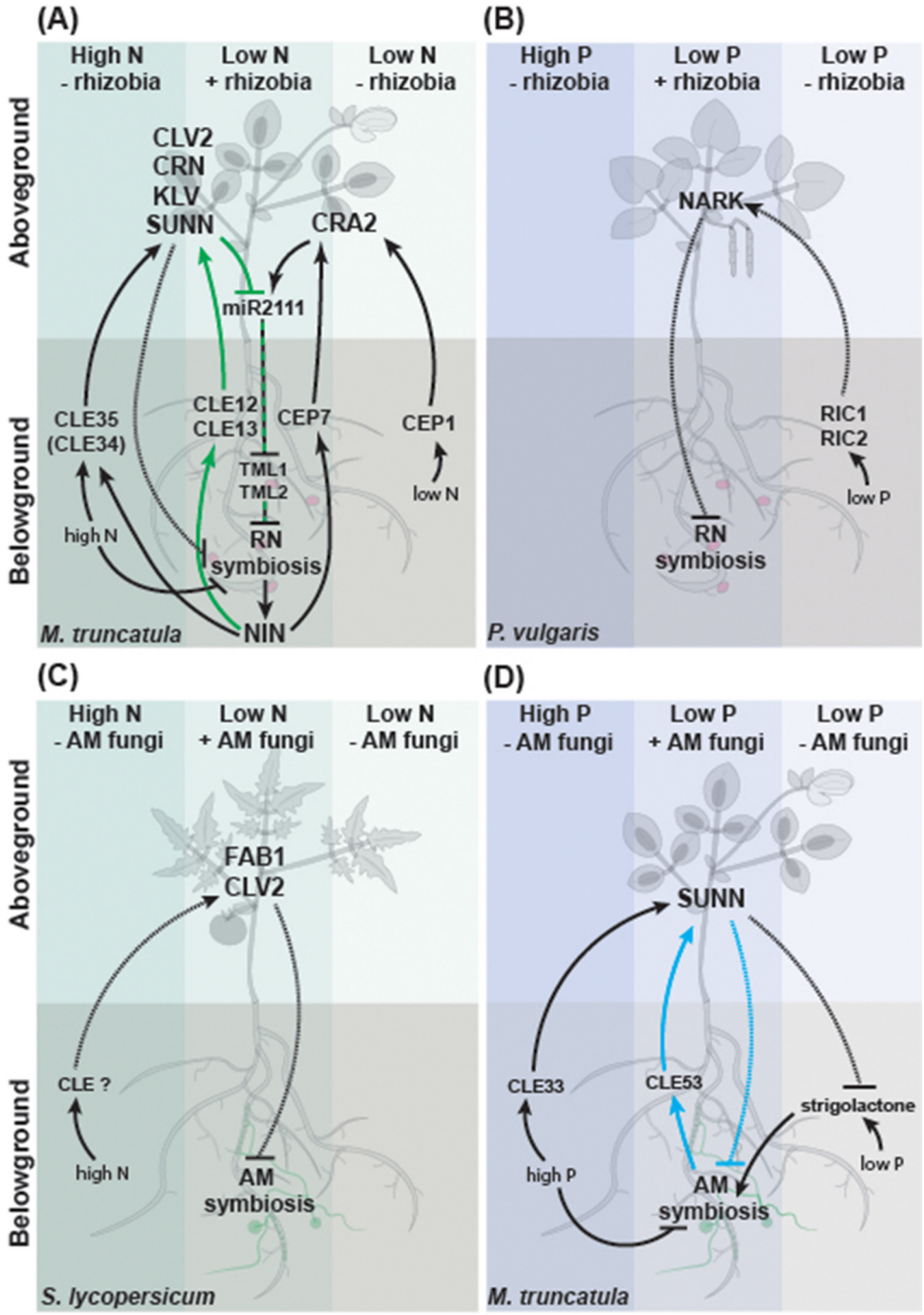
Other signaling peptide classes and environmental signals also participate in plant regulation of symbiosis

Although much less is known, other peptide classes are reportedly involved in RN symbiosis regulation, but their underlying mechanism remains unclear. These include *Mt*RALF1, *Mt*DVL1, *Mt*NRP2, and *Mt*RGF3, all of which negatively regulate nodule number [36–38]. By contrast, the *L. japonicus* PSK pre-propeptide-encoding genes *LjPSK1* and *LjPSK4* were found to be specifically expressed in developing nodules, and application of PSK peptide promoted root growth and nodule development [39]. In addition, short peptides encoded within the open reading frames of miRNAs (e.g., miPep172c and miPep171b [40,41]), within the 5′ upstream regions of protein-coding mRNAs (uORF1p [42]), and in long noncoding RNAs (ENOD40 [43]) were identified as regulators of symbiosis development; however, their molecular function remains largely elusive. It will be interesting to characterize the functional role of these peptides, and to determine whether and how their signaling pathways intersect with CLE and CEP. In addition to the peptides described earlier, which all mediate signaling within the host plant, other peptides, such as nodule cysteine-rich (NCR) peptides, act as **cross-kingdom signals** between the plant and its microbial partners (Box 3).

Furthermore, although most research to date has focused on peptide regulation by symbioses and nutrient status, other environmental signals, such as light, also act through peptides and control the outcome of plant–microbe interactions. Plants with mutations of the transcription factor LATE ELONGATED HYPOCOTYL (LHY) form 50% fewer nodules, each with fewer meristems and lower nodule weight, compared with wild type [44]. A subset of NCR peptides that are expressed rhythmically with the circadian clock have more frequent LHY-bound promoter elements compared with wild type. This suggests that NCR peptides contribute to the integration of light signals with RN symbiosis [44].

Key figure

Partially interconnected systemic peptide signaling pathways regulate symbiosis and nutrient homeostasis



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Two: distinct peptide signaling pathways converge at common downstream signaling hubs

While an increasing number of specific nutrient- or symbiosis-regulated peptide signals is being described, most functional studies to date focus on members of the CLE or CEP family and only a handful of cognate receptors have been identified (Box 2). Interestingly, multiple peptide signals converge at the same receptor (e.g., *M. truncatula* SUNN) and/or trigger common downstream signals, such as the miRNA *miR2111*. In addition to the integration of different symbiosis and nutrient signaling by concerted action of spatiotemporally regulated peptides (see earlier), such integration may also be regulated at the level of signaling cascades acting downstream of the peptide signals.

Perception of many peptide signals converges at one receptor

AON- and AOM-associated CLE signaling pathways converge at shoot-acting receptor complexes characterized by CLAVATA1 (CLV1)-type **leucine-rich repeat receptor-like kinases (LRR-RLKs)**, including *MtSUNN*, *LjHAR1*, *GmNARK*, *PvNARK*, and *Pisum sativum SYM29* (Figure 2A, D and Box 2) [8–10,29,45]. These LRR-RLKs act as negative regulators of RN and AM symbiosis [29,46–52]. Interestingly, this phenomenon does not appear to be only specific to legumes, because *CLV1* orthologs in the non-legumes *Brachypodium distachyon* and tomato were recently reported to act as negative regulators of AM [9,23]. Although no functional connection to AM-induced CLE signaling was demonstrated in these species, these findings suggest that autoregulation of symbiosis is conserved across plant clades.

There is accumulating evidence that *CLV1*-type receptors also integrate nutrient and symbiosis signaling, particularly N and RN signaling (Figure 2A) [26]: plants with mutations of *CLV1*-type LRR-RLKs are defective in suppression of nodule number by high N [53,54], and overexpression of N- and RN-induced *CLE35* in *M. truncatula* reduced nodule numbers in a *MtSUNN*-dependent manner [12,13,15]. However, N regulation of RN symbiosis via *CLV1*-type receptors is dependent on the developmental stage of the nodules, and early (infection thread formation) and late symbiosis stages (N_2 fixation) are regulated via *CLV1*-independent pathways [55–57]. Further research is required to shed light on these, at least partially, distinct regulation patterns; however, it is possible they coincide with spatiotemporal regulation of cognate peptide ligands (Figure 1). In addition, *CLV1*-type LRR-RLKs have a role in the P regulation of RN symbiosis. N_2-fixing nodules are a major P sink and, therefore, nodule formation is inhibited when Pi availability to the plant is low; such inhibition was not observed in *P. vulgaris nark* mutants [58] (Figure 2B).

Figure 2. (A) Autoregulation of nodulation (AON; green) and integration with plant nitrogen (N) status signaling (black). High N induces expression of *MtCLE35* and the pseudogene *MtCLE34*. High N suppression of root nodule (RN) symbiosis depends on the CLV1-type leucine-rich receptor-like kinase (LRR-RLK) *Mt*SUNN but not *miRNA2111*, and *Mt*SUNN-independent pathways also have a role. *MtCLE35* expression is also induced by RN symbiosis via *Mt*NIN. *Mt*SUNN, together with *Mt*CLV2, *Mt*CRN, and *Lj*KLV, is also involved in the perception of the AON regulators *Mt*CLE12 and *Mt*CLE13, which are induced in nodulating roots. Interaction of peptides and receptors in the shoot triggers a downstream signaling pathway involving a phloem-active *miRNA2111* and the F-box proteins *Mt*TML1 and *Mt*TML2, ultimately resulting in suppression of RN symbiosis. RN symbiosis also induces the expression of *Mt*CEP7, which acts as a positive regulator of nodule formation. This signaling pathway involves the shoot-acting LRR-RLK *MtCRA2*. *Mt*CRA2 is also the receptor for N starvation-induced *MtCEP1*, and acts via *miRNA2111*, *Mt*TML1, and *Mt*TML2 to suppress RN symbiosis. (B) Integration of RN symbiosis with plant phosphorus (P) status. *PvRIC1* and *PvRIC2* are induced by low Pi in the absence of rhizobia. The CLAVATA1 (CLV1)-type LRR-RLK *PvNARK* regulates Pi-dependent repression of nodulation in a systemic manner. (C) Arbuscular mycorrhizal (AM) symbiosis integration with N status of the plant. High N-mediated repression of AM symbiosis is dependent on the CLV-type LRR-RLK FAB1 and CLV2 in tomato. It is still unknown whether N-induced CLE peptides have a role in this pathway. (D) Autoregulation of mycorrhizal symbiosis (AOM; blue) and integration with plant P status signaling (black). High P induces the expression of *MtCLE33*, whereas AM symbiosis induces the expression of *MtCLE53*. Both act via the CLV1-type LRR-RLK *Mt*SUNN to suppress strigolactone (SL) biosynthesis and AM symbiosis via as-yet-unknown mechanisms. AM symbiosis is also regulated by P in a SL- and *Mt*SUNN-independent manner. In each panel, the gene names are derived from the plant species for which the most data is available: (A,D) *Medicago truncatula*; (B) *Phaseolus vulgaris*; (C) *Solanum lycopersicum*.

Interestingly, recent evidence suggests that N regulation of AM symbiosis is also regulated by *CLV1*-type LRR-RLKs (Figure 2C): tomato *FAB1* was shown to be involved in N regulation of AM symbiosis, although the cognate N-induced, AM-regulatory CLE peptide remains elusive [23]. The role of *CLV1*-type receptors in AM symbiosis regulation based on P is less clear (Figure 2D): although overexpression of the P-induced *MtCLE33* negatively regulates AM symbiosis biosynthesis in a *MtSUNN*-dependent manner [9], *clv1*-like receptor mutants do not suppress AM inhibition by high P in legumes and non-legumes [9,23,58,59]. This indicates that, in high P conditions, *CLV1*-independent mechanisms are mainly responsible for the suppression of AM symbiosis, although additional layers of regulation via CLE-SUNN modules may exist.

Although multiple distinct CLE signals converge at the same receptor, plants can distinguish between AON- and AOM-associated CLE peptides because overexpression of the RN-associated *MtCLE13* did not affect AM symbiosis [9]. Thus, plants may also be able to distinguish between N- and P-induced CLE peptides. One appealing explanation for such signaling specificity may be that CLV1-type receptors act as common co-receptors in functionally distinct, multiprotein receptor complexes that confer specificity for a particular peptide ligand. A similar mechanism is proposed for CLE signaling in the context of arabidopsis (*Arabidopsis thaliana*) development, in which different receptor complexes comprising combinations of LRR-RLKs (CLV1, BAM1-3, RPK2, and CIK1-4), the receptor-like protein CLAVATA2 (CLV2), and the pseudokinase CORYNE (CRN), are required for CLE perception [60–63]. In legumes, multiple membrane proteins with a function in controlling nodule number in a CLE-dependent manner have been identified. These include the LRR-RLK *Lj*KLAVIER (*Lj*KLV) [64], as well as the *Mt*SUNN interactors *Mt*CLV2 and *Mt*CRN, supporting the hypothesis that they act in the same complex to perceive CLE signals [65]. *CLV2* has also been implicated in regulating AM symbiosis in tomato [66], but not in pea [47], indicating there may be differences in the CLE signal perception complex between legumes and non-legumes. Further research is required to pinpoint the CLE-specific receptor complex compositions that enable symbiosis- and nutrient-specific signaling.

In addition to receptor complex composition, differences in spatiotemporal expression patterns of CLE peptides (see earlier) may also contribute to their signaling specificity. Furthermore, it is conceivable that CLE peptide hormones are likely not the only mobile signals induced by AM or RN symbiosis. Therefore, it is possible that other, parallel pathways contribute to symbiont-specific plant responses.

Distinct receptors activate the same downstream signal cascade

In *M. truncatula,* root-derived and nodulation-promoting CEP1 peptides are perceived by the shoot-acting clade XI LRR-RLK COMPACT ROOT ARCHITECTURE2 (CRA2) [67,68]. *MtCRA2* is a positive regulator of nodule number but does not influence differentiation or metabolic activity, and the analysis of *cra2 sunn* double mutants suggests that the two LRR-RLKs act in separate, antagonistic pathways [67]. Despite these differences, *Mt*SUNN and CRA2 signaling feeds into the same downstream pathway: *MtSUNN* and *LjHAR1* negatively affect the expression of a shoot-to-root-acting miRNA *miR2111* [69,70], whereas *Mt*CRA2 signaling positively regulates the expression of the same *miR2111* [69]. Accumulation of shoot-derived *miR2111* in the roots results in increased competence for nodule formation because the mature form of *miR2111* downregulates the negative nodulation regulators *MtTML1* and *MtTML2* [69–71]. In the context of AM symbiosis or P signaling, the pathway directly downstream of the CLV1 receptor remains elusive. To our knowledge, neither *CRA2, miR2111*, *TML1*, nor *TML2* have been functionally tested for a role in AM symbiosis regulation, but it will be important to establish whether this signaling pathway is specific to RN symbiosis and N homeostasis. One strategy to further our understanding of symbiosis-specific signaling is investigating the processes in non-legume

species that are host plants for AM fungi but not rhizobia. Comparative studies between legumes and non-legumes may allow signaling components to be pinpointed that are specific to one symbiosis or the other.

One receptor triggers different downstream signals

*Mt*SUNN signals through at least three distinct downstream pathways with specific functional outcomes, which may be another way that integration of various signaling inputs is regulated: while overexpression of the nodule-induced *MtCLE13* results in the accumulation of *miR2111*, which in turn results in the degradation of *TML1* and *TML2*, overexpression of the nodule- and nitrate-induced *MtCLE35* also results in the accumulation of *miR2111*, but only *TML2* is degraded while *TML1* levels remain unchanged [12]. Furthermore, additional and potentially *miR2111*-independent shoot-to-root signals acting downstream of *Mt*SUNN have been proposed, including auxin or cytokinin (see later) [72,73]; however, it is not clear whether the two hormones are triggered by distinct CLE peptides. Furthermore, there is evidence that the N-starvation *Mt*CEP1-*Mt*CRA2 module also signals through at least two distinct downstream pathways with specific functions in root development or nodulation [5,19]. In arabidopsis, root system architecture adaptation in response to N starvation is regulated via a systemic feedback loop involving root-derived CEP peptides, two CEP receptors (the *Mt*CRA2 ortholog *At*CEPR1, and *At*CEPR2) in the shoot [6], and phloem-mobile, shoot-to-root-acting CEP DOWNSTREAM polypeptides (*At*CEPD1 and *At*CEPD2) [74]. *Mt*CEP1 and *Mt*CRA2 also regulate root developmental responses to N availability [5,17]; however, in *M. truncatula*, shoot *MtCEPD1* or *MtCEPD2* expression was not responsive to root inoculation with rhizobia [69]. While functional studies are lacking, this indicates that CEPD polypeptides are likely not involved in symbiosis signaling. However, this does not necessarily mean that *miR2111* is the only shoot-to-root symbiosis regulator; similar to what we described earlier for *Mt*SUNN, it is also conceivable that other mobile signals (e.g., classical phytohormones) may act as signals downstream of *Mt*CRA2 in addition to *miR2111* (see later).

The observation that one receptor can trigger multiple different, context-dependent downstream signaling pathways again indicates that interacting proteins, including other (co-)receptors, have a critical role in functional specificity. To disentangle these interconnected signaling pathways, future research may focus on identifying the receptor complex composition or downstream signals specific to each peptide ligand. New methods, including, but not limited to, *in vivo* visualization of peptide–receptor pairs by formaldehyde or photoactivation cross-linking [68], biochemical ligand-binding assays with labeled peptides [6], phosphoproteomics [75], and an increasing wealth of transcriptomic data, will help in this complex quest.

Three: crosstalk between classical hormones and peptide hormone signaling pathways determines symbiosis capacity

Regulation of growth and development by plant hormones is the primary mechanism by which plants coordinate cell division and expansion with different developmental stages and environmental cues. Therefore, studies of hormone crosstalk between peptides and classical hormones provide crucial insights into the regulation of nutrient homeostasis and legume–microbe symbioses. To date, crosstalk between four hormones [auxin, cytokinin, ethylene, and strigolactone (SL)] and peptide signaling has been uncovered. These interactions act to restrict or enhance the extent to which the microbial symbionts are allowed by the host to colonize roots.

CLE-SUNN modules interact with cytokinin, auxin, and strigolactone

There are multiple indications that CEP and CLE signaling interacts with cytokinin signaling. Several studies suggest that AM symbiosis is positively regulated by cytokinin [76]. Furthermore,

cytokinins are both required and sufficient for nodule organogenesis [77]. In *L. japonicus*, overexpression of the AON regulator *LjCLE-RS1* led to an increase in cytokinin biosynthetic intermediates in the shoot, which are reduced in *har1* mutant shoots [73]. Given that shoot-derived cytokinin inhibits nodule formation on *L. japonicus* roots, the hormone was proposed to act as a shoot-to-root signal downstream of *Lj*HAR1 [73]. Root cytokinins also induce the expression of *MtCEP7* and *MtCLE13* dependent on an intact cytokinin signaling pathway downstream of the cytokinin receptor *MtCRE1* [14]. The central regulatory transcription factor NIN, which acts downstream of CRE1, activates transcription of both *MtCEP7* and *MtCLE13* [14], indicating that cytokinin may be a central player in the control of nodule number by fine-tuning expression of the positive regulator *MtCEP7* and the negative regulator *MtCLE13*. In addition, auxin was also proposed to act downstream of *MtSUNN*, because, in *sunn* mutants, shoot-to-root auxin transport was not reduced upon inoculation with rhizobia, as observed in wild-type controls [72,78]. This was a result of increased auxin loading to the phloem rather than differences in the transport capacity, and resulted in elevated auxin levels at the nodule initiation sites in the roots [72], which promotes nodule development possibly by intersecting with cytokinin.

Recent evidence from *M. truncatula* indicates that the CLE-SUNN signaling module intersects with SL signaling in the context of AOM [9]. In *M. truncatula* and other species that are able to form associations with AM fungi, SLs act as signals that are released by roots in response to P deficiency [79]. SLs present in root exudates stimulate the germination of fungal spores by activating their mitochondria and energy production [80,81]. In addition, SLs stimulate the production of mycorrhizal lipochitooligosaccharides, which are perceived by an as-yet-unknown cell surface receptor(s) that triggers the *in planta* AM **common symbiosis signaling pathway (CSSP)**, ultimately resulting in symbiotic phosphate uptake by the transporter PHOSPHATE TRANSPORTER 4 [82,83]. Interestingly, overexpression of the AOM regulator *MtCLE53* resulted in reduced expression of SL biosynthesis genes, such as *DWARF27,* and consequently led to reduced SL production; this effect was abolished in the *sunn* mutant [9]. It was proposed that CLE peptides reduce mycorrhizal colonization by repressing production of an important fungal stimulant, SL, in a *MtSUNN*-dependent manner. However, no significant increase in SL levels or SL biosynthesis gene expression, which could explain the observed hypercolonization in these mutants, was detected either in *sunn* or *clv1*-type mutants of pea (*nark*) and tomato (*fab1*) [9,10,23,84]. This suggests that *MtSUNN* and SL biosynthesis are, at least partially, uncoupled. By contrast, an increase in SL levels was detected in pea *rdn1* mutants [84]. *RDN1* encodes a hydroxyproline *O*-arabinosyltransferase, which is involved in arabinosylation of certain CLE peptides and was initially described as a component of AON [85]. Interestingly, tomato and *M. truncatula rdn1* mutants displayed a hypermycorrhization phenotype similar to *sunn* [10,23], indicating that *RDN1* also acts in AOM. Although the mycorrhizal phenotype of pea *rdn1* has not been experimentally determined, the elevated levels of SL observed in this legume [84] further support a functional, albeit possibly indirect, connection of AOM and SL signaling. Given that SLs are functionally linked to AOM components upstream of the *CLV1*-like LRR-RLK (*MtCLE53, PsRDN1*), but not *MtSUNN/PsNARK/SlFAB1* itself, it is possible that CLE-regulated, CLV1-independent pathways contribute to fine-tuning of AM fungal root colonization via SL.

The CEP1-CRA2 pathway interacts with auxin and ethylene

The *MtCRA2* LRR-RLK is involved in the control of auxin biosynthesis signaling during nodulation in *M. truncatula* [86]: In a comparative transcriptomic study between wild type and *cra2-4* mutants, the authors found that a suite of auxin metabolism, transport, and signaling genes were differentially regulated, including upregulation of the auxin biosynthetic enzyme *MtYUC2.* By contrast, *Mt*CEP1 treatment resulted in downregulation of *MtYUC2* in the wild type but not

in *cra2*. Although the synthetic auxin NAA stimulated lateral root density in the *cra2* mutant background, no functional connection between *Mt*CEP1-*Mt*CRA2 and auxin in the context of nodule number was detected [86]. By contrast, low N-mediated, *Mt*CEP1- and *Mt*CRA2-dependent control of nodule number is regulated via intersection with ethylene signaling: excess ethylene inhibited nodule initiation, an effect that could be partially alleviated by addition of chemically synthesized CEP1 [19]. *Mt*CEP1 control of nodule number requires the *ETHYLENE INSENSITIVE 2/SICKLE (EIN2/SKL)* ethylene response regulator, because *Mt*CEP1 application cannot further increase the nodule number in the hypernodulating *skl* mutant, whereas in *sunn* it still can [19]. Interestingly, biochemical experiments showed that *Mt*CRA2 can directly transphosphorylate *Mt*EIN2 at the Ser643 and Ser924 positions, two conserved phosphorylation sites required for EIN2 signaling in arabidopsis [86]. Phosphorylation of *Mt*EIN2 stabilizes the protein and prevents activation of the ethylene signaling pathway, which consequently allows rhizobial infection to occur [86].

Taken together, accumulating evidence suggests that classical phytohormone and peptide hormone signaling pathways are tightly interconnected, and influence each other directly or indirectly. This link potentially allows integration of multiple signals across cellular and tissue scales, and a combined action of phytohormones and peptides may contribute to functional specificity of peptide signaling.

Four: peptides with specialized roles during symbiosis are often members of large families with evolutionary conserved function in plant development

The ancient and widespread AM symbiosis is thought to be 'the mother of all endosymbioses', with multiple genes shared with the relatively younger RN symbiosis clade; species with the ability to establish RN symbiosis are variously distributed among the Fabales, Fagales, Cucurbitales, and Rosales orders [87,88]. Molecular phylogenomic studies suggest that both AM and RN symbioses were independently lost several times during angiosperm evolution [89,90]. However, the ability to form either symbiosis correlates with an expansion of many gene families required to refine associations with microorganisms [88,90]. The CLE and NCR peptide families are the largest family of peptides encoded within legume genomes, such as that of *M. truncatula* [20]. While CLEs are not only present in legumes but across all evolutionary clades, the presence of NCRs is restricted to galegoid legumes of the Inverted Repeat Lacking Clade, which require rhizobia to undergo terminal differentiation before N fixation (Box 3). The presence of CLE peptides even in nonmycorrhizal, early-diverging land plants indicate that their ancient function is the regulation of plant development (Figure 3) [91]. However, when expressed during AM or RN symbiosis, some CLE peptides have specific roles to limit symbiosis progression [8,9]. Conversely, NCR peptides, such as the closely related defensin peptides, have nonsymbiotic antimicrobial activities and, if not correctly processed in nodules, N fixation is arrested (Box 3) [92,93]. Therefore, has peptide activity neofunctionalized in legumes over the course of evolution or were these peptides recruited into the symbiosis pathways from more ancient root developmental pathways? To date, there is more evidence to support the latter hypothesis rather than the acquisition of new roles during evolution, although NCR peptide evolution remains a conundrum.

Phylogenetic relatedness of CLE pre-propeptides may be predictive of function during nutrient deficiency and symbiosis

The legumes *M. truncatula* and *L. japonicus* encode 53 and 52 CLE pre-propeptides, respectively, slightly more than the 32 encoded in the nonnodulating and nonmycorrhizal arabidopsis but less than AM-competent cereals, such as wheat [94]. Within legumes, however, CLE peptides with a function in AM or RN symbiosis (e.g., AON-associated *Mt*CLE12 and *Mt*CLE13, rhizobia- and N-induced *Mt*CLE35, or the AOM regulator *Mt*CLE53) are phylogenetically closely

related [95–97]. N- and P-induced CLEs (e.g., *Gm*NIC or *Mt*CLE33) fall in related but distinct clusters [94,97]. At the sequence level, a conserved five-amino acid residue sequence TLQAR within the signal peptide domain, predicted to be the cleavage site, defines nodulation-suppressing CLEs, such as *Mt*CLE35 [94]. Nitrate-induced *MtCLE35* and *MtCLE34* are present in tandem on chromosome 2 of the *M. truncatula* genome, suggesting that they arose due to a gene duplication event and that *MtCLE34* was lost due to pseudogenization [15]. In addition to their *sunn*-dependent roles in AON and AOM, when externally applied to plants, synthetic *Mt*CLE12 and *Mt*CLE13 retained symbiosis-unrelated morphogenic properties and affected plant development, just like other CLE peptides described in arabidopsis; however, validation of the biological activity of the peptide species encoded *in vivo* will lend more credibility to this finding [8].

Alteration of bacteroid shape by NCR peptides may provide evolutionary advantages

NCR peptides (Box 3) are only found in inverted repeat-lacking clade (IRLC) legume nodules, wherein rhizobia are terminally differentiated and cannot be reisolated and/or cultured as free-living bacteria. Terminally or irreversibly differentiated bacteria can only be observed in legume nodules with a persistent meristem called 'indeterminate' type nodules, such as in *M. truncatula*. In 'determinate' nodule-forming plants, such as *L. japonicus*, *G. max*, and *P. vulgaris*, NCR peptides are absent, and bacteria do not differentiate. Although NCRs have antimicrobial activities, within nodules they are targeted to the symbiosome membrane, where they act at sublethal concentrations and mediate their effects on bacterial differentiation [98]. Interestingly, the size of the peptide family is directly related to the degree of bacterial differentiation in a given host plant [99]. For example, terminally differentiated bacteria in *M. truncatula* nodules are four times larger than their free-living counterparts compared with the bacteroids in *Cicer arietinum,* the size of which increases less than twofold relative to free-living rhizobia [100]. This correlates with the total number of NCR peptides in *M. truncatula* (639) versus 63 in *C. arietinum*. Both local gene duplications and/or whole-genome duplications are thought to have caused the expansion of NCRs in certain legumes [99,101]. Curiously, although lupin (*Lupinus albus*) forms nodules similar to indeterminate-type ones, and has swollen bacteroids, so far, no NCRs have been identified in its genome (Figure 3) [102]. The NCR peptides originated, expanded, and are still retained, in legume genomes; therefore, they likely provide some evolutionary advantage. Studies in *Aeschynomene* species, legumes belonging to the dalbergioid clade, which has a nod factor-independent mechanism of interaction with photosynthetic bradyrhizobia, show that NCR-Like peptides encoded within their genome can induce formation of spherical bacteroids, which correlate with a higher N-fixation capacity compared with elongated bacteria [103,104].

Peptides with roles in root development were co-opted into root nodule symbiosis

An analysis of select peptide families with known roles in AM or RN symbiosis, namely CLE, CEP, RGF, PSK, and NCR, across major plant taxa, supports the hypothesis that legume peptides have retained a subset of their original ancestral function [105,106]: Similar to the CLE peptides, CEPs also function during plant development, regulate root system architecture in response to N availability, and are encoded within angiosperm and gymnosperm genomes, but are not detected in other ancient plant lineages (Figure 3) [6,17,19,74,107,108]. Another family of root growth regulators are the RGF peptides, which are present in all root and rhizoid-forming bryophytes and plants but absent in green algae and the only sequenced lycophyte (Figure 3) [105,109]. This suggests an evolutionarily important role in a shift to a land-based habitat consistent with the roles of RGFs identified to date, including gravity sensing, lateral root initiation and emergence, interaction with plant pathogens, and a role in nodule formation [110–112]. Nodule and lateral root development are both regulated by similar developmental programs [113,114]; therefore, it is not surprising that peptide families such as CLE, CEP, or RGF, with important roles during root development, were co-opted to regulate the developmental programs associated with nodule formation

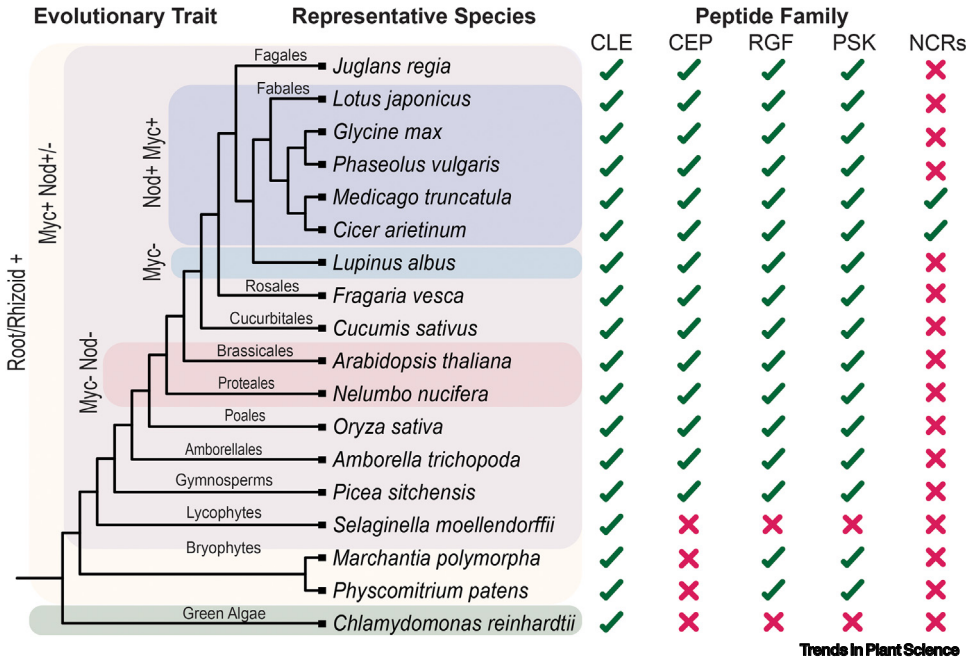

Figure 3. Phylogenetic tree showing the presence–absence of peptide families in different plant species with known roles in nodulation and mycorrhization. As photosynthetic organisms moved from water to land, they had to adapt to a nonmotile lifestyle. To do so, ancestors of land plants acquired root-like organs to take up nutrients from their surroundings and for anchorage. These root-like structures are present in early-diverging plant lineages, such as bryophytes (*Physcomitrium patens* and *Marchantia polymorpha*) and lycophytes (*Selaginella moellendorffii*). Over time, roots became the primary organs providing a surface for interactions with beneficial soil microbes, such as mycorrhizae. The ability to form symbiotic associations with mycorrhizae is thought to be evolutionarily ancient since ~70% of all land plants can form arbuscular mycorrhizal (AM) symbioses. Interestingly, this ability was lost in members of Brassicaceae (*Arabidopsis thaliana*), Proteaceae (*Nelumbo nucifera*) and a few others. As legumes and members of the Fabales, Fagales, Cucurbitales, and Rosales acquired the ability to nodulate in association with rhizobia, they recruited many genes involved in AM symbiosis and lateral root development into the process of nodulation. *Lupinus albus* remains the only known legume that can nodulate, but not form associations with, mycorrhizae. Please note that gymnosperms such as *Picea spp.* associate with ectomycorrhizal fungi and other species in the *Marchantia* genus such as *M. paleacea* act as AM hosts. Adapted from [106].

[113,114]. Alternatively, an increase in the number of LRR-RLKs in angiosperms compared with basal land plants might also help explain how peptide signaling became more specialized (Figure 3) [105]. Further investigation of receptor evolution and functional characterization might provide more insight into this question.

Five: microbial peptide mimics alter host physiology to enhance colonization

Molecular mimicry has been observed widely among plant-associated pathogens. Mimicry refers to the production of microbial products that are identical in structure and chemistry to host plant metabolites and confer some evolutionary advantage to the pathogen by increasing their virulence [115]. These microbial products, or 'mimics', are recognized by plant receptors as bona fide signals, thereby activating nutrient uptake, root developmental, or immunomodulatory pathways downstream of the host-encoded peptide signal [116]. For instance, RALF-like peptides were discovered in the fungal plant pathogen *Fusarium oxysporon* [117], and the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* was shown to secrete proteinaceous effectors mimicking PSY [118]. In addition, peptides resembling CLE, CEP, and RALF have been identified in root-knot (e.g., *Meloidogyne incognita*) or cyst nematodes (e.g., *Heterodera glycines*) [116,119–121]. One of the best studied examples is the parasitic interaction between cyst-forming nematodes that produce CLE peptides to activate uncontrolled cell division in the vascular tissue at nematode

feeding sites called syncytia, which ensures that the female larvae derive sufficient nutrients from these cysts on soybean roots to complete one reproductive cycle [122]. It is interesting to note that functional CLE peptides are post-translationally arabinosylated and/or hydroxylated, although no post-transcriptional modification machinery has been identified in nematodes [126]. Therefore, these microbes therefore likely exploit the plant post-translational modification machinery to improve their own chances of survival.

Pathogens and plant parasites are thought to have developed effectors and host peptide mimics in a constant arms race for survival [115]. In the context of symbiosis, plants have evolved an elaborate machinery to invite and accommodate beneficial microbes [2,123]. Is it then reasonable to hypothesize that symbiotic fungal or bacterial genomes also encode host-modulating peptides? Thorough bioinformatic searches have identified many fungal peptides in both ectomycorrhizal and AM fungi that are induced under symbiotic conditions [124,125]. The AM fungi *Rhizophagus irregularis* and *Gigaspora rosea*-encoded CLE-like peptides, *Ri*CLE1 and *Gr*CLE1, are induced in colonized roots of *M. truncatula* but not in fungal spores or hyphae alone [21]. The *Ri*CLE1 peptide shares high homology with *Mt*CLE05, which is induced by low soil P levels [16,21]. Application of synthetic *Ri*CLE1 or *Mt*CLE05 stimulates lateral root production in *M. truncatula* [16,21]. Moreover, pretreatment with synthetic *Ri*CLE1 increased mycorrhizal colonization by increasing the number of fungal entry points [21]. Therefore, it is possible that fungal CLE peptide mimics help simulate low P conditions, which in turn stimulates lateral root formation and AM fungal colonization, and, thus, confer an evolutionary advantage to AM fungi by enhancing mycorrhizal infectivity [21]. Although root system architecture response to *Ri*CLE1 treatment was partially dampened in arabidopsis and pea *clv2* (but not *clv1*) mutants, the *in planta* receptors remain to be identified. To date, no plant peptide mimics have been reported in rhizobial genomes. Bioinformatics-based reannotation pipelines and sequence-based analyses have been instrumental in identifying previously overlooked genes encoding short signaling peptides [20]; thus, a systematic reannotation of bacterial genomes might help identify plant mimetic peptide candidates within rhizobia and/or other bacterial partners.

Concluding remarks

As we begin to grasp the emerging principles underlying peptide control of symbiosis and nutrient acquisition, one thing is clear: peptide hormones are potent growth regulators that bear great potential for biotechnological applications in agriculture. Effects of synthetic peptides, when applied as seed coatings or drench treatments, on nutrient uptake, disease resistance, and RN and AM symbiosis are promising and showcase the potential for such peptide applications to boost symbiotic capabilities in the field [7,21,40,41,127]. While it remains to be determined whether seed priming with peptides can withstand relatively harsher conditions in soil, tolerate the presence of soil microbes that use peptides as a nutrient source, or whether their application translates into enhanced crop yields; the research community has laid the groundwork for technological advancement that will likely benefit agricultural productivity in the coming decades (see Outstanding questions).

Outstanding questions

What is the mechanistic role of understudied peptide classes, such as RGF or PSK, in symbiosis regulation?

Are there any peptides that evolved in legumes specifically for interactions with symbiotic microbes? Conversely, did rhizobia evolve any plant peptide mimics to boost their infectivity and/or ability to evade host defense?

How is peptide–receptor specificity achieved in the context of symbiosis and nutrient signaling? Are there peptide–receptor pairs that participate in both AM and RN symbiosis?

What role does differential post-translational modification have in peptide function? Are there additional, yet unidentified, post-translational modifications that can modulate the activity of peptides? Are there symbiosis-regulated enzymes that control processing and post-translational modifications of peptides during host–microbe interactions?

Can we manipulate peptide signaling pathways to increase symbiotic capacities in crops?

What is the significance of peptide-signaling pathways under field conditions?

Do central symbiotic regulators, such as NIN, act to integrate multiple peptide signals?

Declaration of interests

None declared by authors.

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