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The Function of the CLE Peptides in Plant Development and Plant-Microbe Interactions

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The CLAVATA3 (CLV3)/ENDOSPERM SURROUNDING REGION (ESR) (CLE) peptides consist of 12 or 13 amino acids, including hydroxylated proline residues that may or may not contain sugar modifications, and function in a non-cell-autonomous fashion. The *CLE* gene was first reported in *Zea mays* (maize) as an endosperm-specific gene, *ESR*, in 1997 (Opsahl-Ferstad et al., 1997). *CLE* genes encode secreted peptides that function in the extracellular space as intercellular signaling molecules and bind to cellular surface receptor-like proteins to transmit a signal. CLE peptides regulate various physiological and developmental processes and its signaling pathway are conserved in diverse land plants. Recent CLE functional studies have pointed to their significance in regulating meristematic activity in plant meristems, through the CLE-receptor kinase-WOX signaling node. *CLV3* and *CLE40* are responsible for maintenance of shoot apical meristem (SAM) and root apical meristem (RAM) function, regulating homeodomain transcription factors, *WUSCHEL* (*WUS*) and *WUSCHEL-related homeobox 5* (*WOX5*), respectively. CLE and WOX form an interconnected and self-correcting feedback loop to provide robustness to stem cell homeostasis. CLE peptides are required for certain plant-microbe interactions, such as those that occur during legume symbiosis and phytopathogenic nematode infection. Understanding the molecular properties of CLE peptides may provide insight into plant cell-cell communication, and therefore also into plant-microbe interactions.

INTRODUCTION

Multicellular organisms rely on cell-cell communication for their development, growth, and environmental responses. For this purpose, plants and animals utilize secreted signaling molecules, such as peptide hormones. These signaling molecules are produced by one cell and perceived by the corresponding cell surface receptors on neighboring cells, and elicit an orchestrated intracellular signaling cascade that often leads to transcriptional reprograming and the appropriate adjustment of conditions within the cell. Thus, multicellular organisms achieve organized development. The animal peptide hormone insulin, which regulates fat and steroid metabolism, was identified in 1922. Systemin was the first plant peptide hormone to be identified, and was shown to function in the wounding response in 1991 (Pearce et al., 1991). Thus, the field of plant peptide hormone research is relatively young.

In Arabidopsis, *CLV3* was first found to encode a small extracellular protein that regulates the number of cells in the shoot apical meristem (SAM) in a non-cell-autonomous fashion (Fletcher et al., 1999). The mature structure of the CLV3 peptide was reported to be a dodecapeptide in 2006 (Kondo et al., 2006). Further, some of the CLE peptides, including CLV3, were found to function as arabinosylated glycopeptides (Fig. 1; Ohyama et al., 2009; see Matsubayashi, 2011).

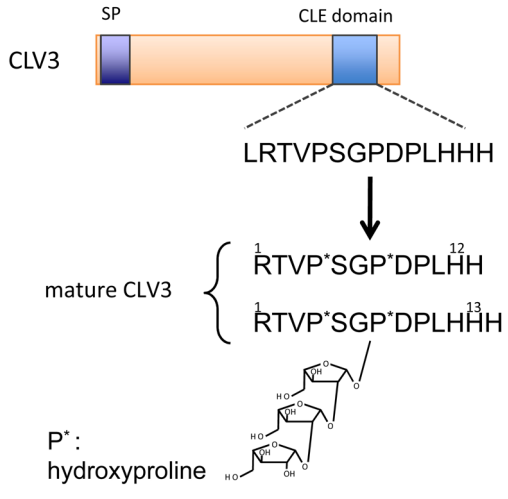

Figure 1. The molecular structure of mature CLV3 peptides, as an example of a CLE protein.

A schematic model of CLV3, showing the N-terminal signal peptide (SP) and C-terminal CLE domain. The amino acid sequence of the CLV3 CLE domain is given below the CLE domain. The two mature forms of CLV3 that have been detected in plant tissue are also shown at the bottom. Hydroxylated proline residues are indicated by asterisks.

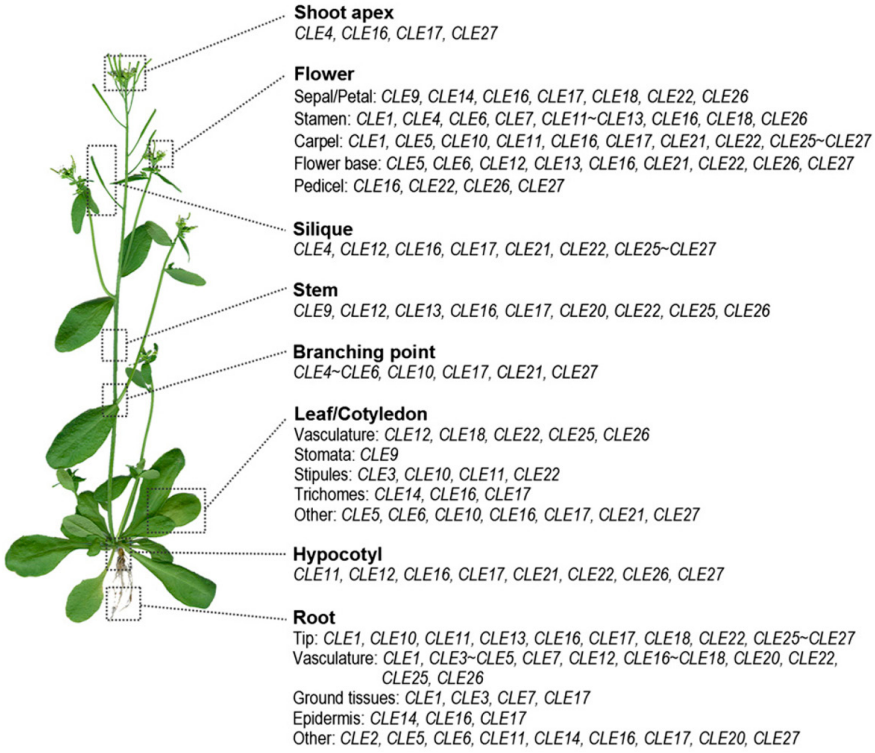

Figure 2. Summary of A-type CLE promoter activity in Arabidopsis. Shown is a list of the CLE genes expressed in the various tissues of a mature Arabidopsis plant. Image reprinted from Jun et al. (2010).

Arabidopsis harbors 32 *CLE* genes (Fig. 2 and Table 1). Among them, *CLV3* and *CLE40* are responsible for the maintenance of the SAM and the functioning of the root apical meristem (RAM), and they achieve this by regulating the expression of the homeodomain transcription factors, *WUSCHEL* (*WUS*) and *WUSCHEL-related homeobox 5* (*WOX5*), respectively (Fig. 3a). The CLE-WOX signaling partners form an interconnected and self-correcting feedback loop that maintains stem cell homeostasis in the SAM and RAM. *CLE41* and *CLE44* encode TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR (TDIF) regulating *WUSCHEL-related homeobox 4* (*WOX4*), which contributes to the balanced maintenance of vascular stem cells (Fig. 3a; see Matsubayashi, 2011).

A number of cell surface receptors have been identified to be required for perception of CLE peptides. Three major receptor kinase complexes of CLV1, CLV2-SUPPRESSOR OF LLP1-2 (SOL2)/CORYNE (CRN), and the recently identified RECEPTOR-LIKE PROTEIN KINASE 2 (RPK2)/TOADSTOOL 2 (TOAD2) are required to transmit the CLV3 signal in the SAM (Fig. 3a and Table 2; Clark et al., 1995; Kayes and Clark, 1998; Sawa, 2006; Sawa and Tabata, in press; Tabata and Sawa, in press). CLE40 is recognized by the receptor kinase ARABIDOPSIS CRINKLY4 (ACR4) and regulates *WOX5* expression in the RAM (Stahl et al., 2009). In the vascular meristem, TDIF RECEPTOR/ PHLOEM INTERCALATED WITH XYLEM (TDR/PXY), which has a high level of sequence similarity to CLV1, perceives the TDIF signal from phloem to regulate *WOX4* expression (Fig. 3a and Table 2; Hirakawa et al., 2008; Hirakawa et al., 2010b). Thus, the CLE-receptor kinase-WOX module appears to be conserved in these three meristems: SAM, RAM and vascular cambium. Furthermore, this CLE-receptor kinase-WOX module that regulates meristem activity appears to be conserved in grasses, rice and maize (Opsahl-Ferstad et al., 1997; Taguchi-Shiobara et al., 2001; Suzaki et al., 2004; Bommert et al., 2005; Suzaki et al., 2006; Suzaki et al., 2008; Suzaki et al., 2009).

Besides its involvement in maintaining the meristem, CLE activity is indispensable for certain plant-microbe interactions. In leguminous plants, the CLV1 homologues, *HYPERNODULATION ABERRANT ROOT* (*HAR*) *1*, *SYM29*, *NITRATE TOLERANT SYMBIOTIC* (*NTS*)*-1,* and SUPER NUMERIC NODULES (*SUNN*) have been reported to negatively regulate the number of newly produced nodules (Fig. 3b and Table 2; Krusell et al., 2002; Nishimura et al., 2002; Searle et al., 2003; Schnabel et al., 2005). This inhibitory signal is proposed to arise in developing nodules in the roots and is further transmitted through the shoot to regulate the formation of nodules in the roots (Kosslak and Bohlool, 1984). The recent identification of the CLEs involved in this autoregulation of the nodule as candidate mobile signals emphasizes the possibility that plants might utilize CLE peptides not only as short-distance signals but also as long-distance mobile signals (Fig. 3b; Okamoto et al., 2009; Mortier et al., 2010).

Surprisingly, the CLE-like sequence is also found in phytoparasitic nematodes, such as root-knot nematodes and cyst nematodes (Wang et al., 2001; Huang et al., 2006b; Davis et al., 2008;

Table 1

CLE peptide classification based on the effectiveness

Group	Member	SAM RAM	Protoxylem	Xylem (*Zinnia*)
Group-A	CLV3	+	+	–
	CLE8	+	+	–
	CLE9/10	+	+	–
	CLE11	+	+	–
	CLE12	+	+	–
	CLE13	+	+	–
	CLE14	+	+	–
	CLE16	+	+	–
	CLE17	+	+	–
	CLE18	+	+	–
	CLE19	+	+	–
	CLE20	+	+	–
	CLE21	+	+	–
	CLE22	+	+	–
	CLE27	+	+	–
	CLE40	+	+	–
	CLE45	+	+	–
Group-B	CLE25	+	–	–
	CLE26	+	–	–
Group-C	CLE41/44	–	–	+
	CLE42	–	–	+
Group-D	CLE1/3/4	–	–	–
	CLE2	–	–	–
	CLE5/6	–	–	–
	CLE7	–	–	–
	CLE46	–	–	–
	CLE43	N/D	N/D	N/D

+, effective; –, ineffective; and N/D, not determined.

Table reprinted from Hirakawa et al. (2011) with permission from Elsevier.

Mitchum et al., 2008). Both nematodes are capable of developing highly specified organs, called giant cells and syncitia, respectively, for their parasitic life cycles in the host roots (Davis et al., 2008; Mitchum et al., 2008). Accumulating evidence suggests that these nematodes might hijack the plant innate CLE signaling system to secure their parasitic lifestyles (Fig. 3a; Wang et al., 2005; Huang et al., 2006b; Davis et al., 2008; Mitchum et al., 2008; Patel et al., 2008).

The *CLE* gene family occurs in various plants, including the green alga, *Chlamydomonas reinhardtii*, although the amino acid sequence of the *Chlamydomonas* CLE precursor is not well conserved (Oelkers et al., 2008; Miwa et al., 2009a). With the exception of *Chlamydomonas reinhardtii*, the *CLE* genes were found only in multicellular plants and phytoparasitic nematodes. CLE precursor proteins of the moss, *Physcomitrella patens,* also have a signal peptide sequence that directs them to the extracellular space, and these proteins seem to function as peptide hormones.

In this chapter, we will summarize recent advances in our understanding of CLE activities in plants. In addition to the well-documented activities of CLV3 and TDIF, we also focus on CLE activities in plant-microbe interactions, such as legume symbiosis with rhizobial bacteria and plant-phytoparasitic nematode interactions, both of which might have developed from the endogenous CLE signaling pathway that regulates morphogenesis in plants. We will present a comprehensive overview of CLE functions in organisms beyond Arabidopsis in an effort to improve our understanding of the significance of CLE activity in plants,

THE CLV3/ESR FAMILY

The *CLE* gene family encodes small (~100 amino acids long) proteins that have a conserved structure that includes a putative N-terminal secretory signal peptide and a conserved 14-amino acid CLE domain at the C-terminus (Fig. 1; Cock and McCormick, 2001; Sharma et al., 2003; Sharma et al., 2005; Strabala et al., 2006; Jun et al., 2008; Sawa et al., 2008; see Matsubayashi, 2011). Apart from the amino acid sequences within the CLE domain, the amino acid sequences of CLE proteins are highly diverged. Domain-swap and deletion experiments based on *CLV3* suggest that the signal peptide and CLE domain are crucial for the functioning of the encoded protein, indicating that CLE peptides must be secreted and that the CLE domain is primarily responsible for CLV3 activity *in planta* (Rojo et al., 2002; Fiers et al., 2006; Ni and Clark, 2006). Indeed, biochemical studies have identified 12- or 13-amino acid peptides encompassing the CLE domain *in planta* (Fig. 1, Ito et al., 2006; Kondo et al., 2006; Ohyama et al., 2008; Ohyama et al., 2009). In addition, application of chemically synthesized CLV3 peptides corresponding to the CLE domain induced termination of SAM and RAM in Arabidopsis, a phenotype resembling that of the *CLV3*-overexpression (Fiers et al., 2005; Fiers et al., 2006; Ito et al., 2006; Kondo et al., 2006). The smallest unit exhibiting CLV3 activity was found to be a 12-amino acid peptide, RTVPSGPDPLHH, namely MCLV3 (Fig. 1, Kondo et al., 2006; see Matsubayashi, 2011). These data suggest that CLV3 functions i*n planta* as a secreted peptide ligand derived from the CLE domain.

The functional relevance of the CLE domains was further supported by the identification of TDIF from the *Zinnia elegans* cell culture system as a 12-amino acid peptide corresponding to the CLE domains of Arabidopsis CLE41 and CLE42 (Ito et al., 2006). To gain insight into the role of the remaining CLE peptides in Arabidopsis development, the12-amino acid forms of 26 Arabidopsis CLE peptides corresponding to the CLE domains of the 32 CLE proteins encoded in the Arabidopsis genome were chemically synthesized and examined using Arabidopsis plants (Ito et al., 2006; Hirakawa et al., 2011). The results demonstrated that Arabidopsis CLEs can be divided into four groups based on their activities when applied as 12-amino acid synthetic peptides. The first group suppresses apical meristem development and protoxylem vessel cell differentiation; the second exhibits only meristem-suppressing activity; the third exerts protoxylem vessel cell differentiation; and the fourth has no apparent plant growth phenotype (Table. 1; Hirakawa et al., 2011).

Biochemical studies of the CLE peptides also identified some of them as being post-translationally modified (see Matsubayashi, 2011). The fourth and seventh proline residues are often hydroxylated, although the contribution of hydroxylation to CLE activity remains unclear (Fig. 1; Ito et al., 2006; Kondo et al., 2006; see Matsubayashi, 2011). Hydroxylation itself is not important for the conformation and bioactivity of CLE. However, hydroxylation provides the scaffold that is required for CLE pep-

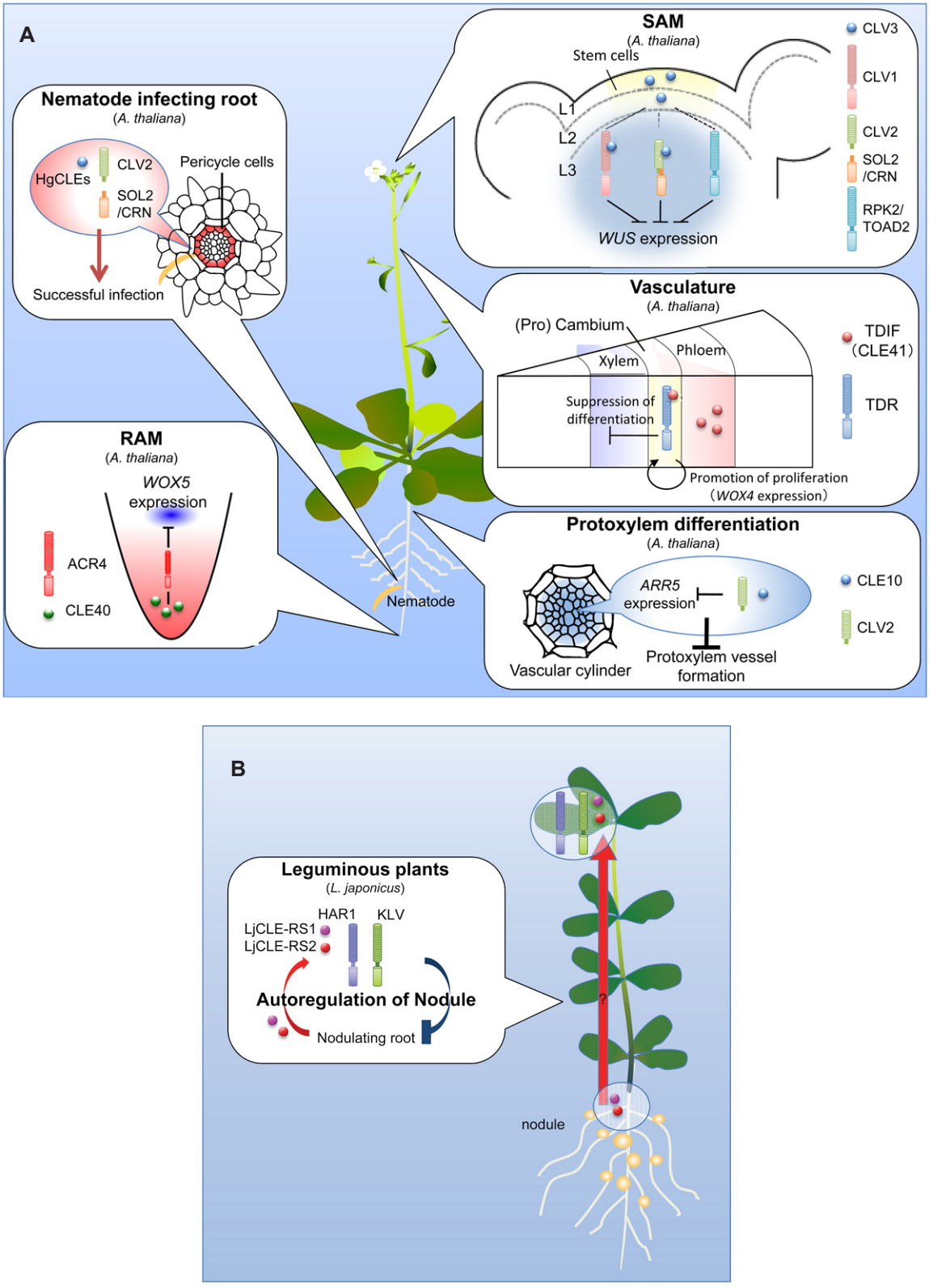

Figure 3. CLE activities and receptors in plants.

(A) CLE activities based on studies using Arabidopsis. **(B)** The role of CLE in nodulation of leguminous plants. CLE activities in various aspects of plant development and of plant-microbe interactions are schematically depicted together with their corresponding receptors. Direct CLE-receptor pair associations are depicted by direct binding between the schematically drawn CLE and receptors. Lj, *Lotus japonicus*; Mt, *Medicago truncatula*; Hg, H*eterodera glycines*.

Table 2. A list of the known CLE-receptor combinations and their roles in various plant activities.

CLE	Receptors	Function
CLV3	**CLV1, CLV2**-CRN/SOL2, RPK2	Maintenance of the SAM
CLE40	ACR4	Cell layer organization in the RAM
CLE41	**TDR**	Vascular stem cells maintenance
CLE10	CLV2	Protoxylem vessel formation
LjCLE-RS1, LjCLE-RS2	HAR1, KLV	Autoregulation of nodulation
LjCLE-RS2	HAR1, KLV	Nitrate-dependent regulation of nodulation
MtCLE13	SUNN	Autoregulation of nodule, Petiole length control
HgCLE1	CLV2-CRN/SOL2	Cyst nematode infection

Genetically and biochemically identified CLE-receptor combinations are shown. The receptors in bold letters indicate that their interactions to the cognate CLE peptides have been demonstrated not only genetically but also by biochemical means. Please refer to the text for the corresponding references.

tide glycosylation (Kondo et al., 2011a). Post-translational arabinosylation was found to occur on the seventh proline residue of several CLE peptides, including CLV3 (Fig. 1; Ohyama et al., 2009; see Matsubayashi, 2011). The arabinosylated form of CLV3 exhibits a greater tendency to terminate the SAM of Arabidopsis than the non-arabinosylated form, probably because the glycosylated form of CLV3 has an enhanced affinity for its receptor, CLV1 (Ohyama et al., 2009). Therefore, the previously observed hydroxylation of proline residues might be required for the subsequent arabinosylation of the CLE peptides. It remains unclear how common sugar modification is among the CLE peptides, due to the technical difficulties in extracting fully intact CLE peptides from plant tissues and in synthesizing arabinosylated peptides in vitro. However, the possible contribution of sugar modification to CLE activity should be noted.

The variable domains of CLE proteins between the signal peptide and CLE domain are thought to be dispensable, based on a number of studies that focus on CLV3 activity (Kondo et al., 2006; Ni and Clark, 2006). However, a recent study shows that the variable domains of some CLE proteins contribute to CLE function (Meng et al., 2010). Domain-swap experiments, for example, between CLE14 and CLE1, demonstrated that a fusion of the CLE1 variable domain with the CLE domain of CLE14 resulted in increased termination of the SAM relative to the native CLE14 variable domain when overexpressed in Arabidopsis (Meng et al., 2010). Other chimeras were also shown to decrease SAM termination (Meng et al., 2010). Interestingly, a putative signal peptide sequence was also found to contribute to CLE activity, since substituting the signal peptide sequences of CLE6, CLE7, and CLE20 with one of the rice glycine-rich cell wall structural protein 2 precursors resulted in decreased activity relative to the native versions of CLE6, CLE7, and CLE20 when overexpressed in Arabidopsis (Meng et al., 2010). Thus, sequences outside the CLE domain, including putative signal sequences, contribute somewhat to the function of some CLE proteins.

The identification of mature forms of CLE proteins *in planta* suggests that polypeptide processing machinery is involved in the generation of CLE peptides (Ito et al., 2006; Kondo et al., 2006). If this is indeed the case, the sequences outside of the CLE domain might provide recognition specificity for peptidases, and this might be required for expressing the specific activity of some CLE peptides (Meng et al., 2010).

CLE protein-processing activity was reported in the extracts of Arabidopsis inflorescences and leaves, and in the medium of BY2 cell cultures (Ni et al., 2011). The authors suggested that the putative protease responsible for this processing activity would recognize four residues upstream of the CLE domain; however, the protease has not yet been isolated.

A genetic suppressor screen for the short-root phenotype induced by the root-specific expression of *CLE19* in Arabidopsis identified *Suppressor of LLP1* (*SOL*) *1*, which encodes a Zn^{2+} carboxypeptidase that is predicted to function in the processing of CLE proteins (Casamitjana-Martinez et al., 2003). However, besides *CLE19*, none of the other *CLE* genes were examined for a dependency on *sol1*. Thus, a detailed functional and biochemical analysis of SOL1 might provide novel insight into the CLE processing machinery.

The structure of *CLE* genes is well-conserved; however, some *CLE* genes, for instance those found in rice and *Medicago truncatula,* encode CLE proteins with multiple tandemly arranged CLE domains (Kinoshita et al., 2007; Oelkers et al., 2008; Sawa et al., 2008; Mortier et al., 2010). The yeast peptide pheromone, alpha factor, carries four tandem repeats of 13-amino acid peptides in the precursor and, upon translation of the precursor, the KEX2 endopeptidase cleaves the alpha factor from the precursor, which is thought to promote a massive induction of the pheromone (Dmochowska et al., 1987). Thus, CLE proteins with multiple CLE domains might be subjected to similar steps to generate mature CLE peptides (Sawa et al., 2008).

THE ROLE OF CLE IN MAINTAINING SAM HOMEOSTASIS

Structure and function of SAM and CLV3-WUS feedback loop

The SAM is a collection of cells that continuously renews itself by cell division and supplies cells for new organs. Although cells continuously divide and differentiate in meristematic regions, the meristem size and the number of stem cells remain constant. These observations suggest that stem cell maintenance and new organ formation are well balanced.

The SAM can be divided into three layers, based on cell fate (Fig. 3a). Cells of the L1 and L2 layers divide in an anticlinal orientation, perpendicular to the plane of the meristem, and produce epidermal cells and mesophyll cells, respectively. Other cells and tissues are produced from the L3 layer. Furthermore, the SAM is divided into three zones, the peripheral zone (PZ), the central zone (CZ), and the rib zone (RZ). Multi-potent stem cells are located in the CZ, and the rate of cell division is relatively low in this zone. However, cells in the RZ divide frequently to give rise to new lateral organs, leaves, and flowers (Miwa et al., 2009b).

CLV3 expression is observed in the stem cells, mainly in the L1 and L2 layers of the CZ (the region of the SAM highlighted in yellow in Fig. 3a). The CZ of the *clv1*, *clv2*, and *clv3* mutants is enlarged. However, the size of the RZ was almost identical to that of wild-type plants, and the size of lateral organs, including leaves and flowers, was also normal (Clark et al., 1995). Live imaging techniques revealed that CLV3 restricts its own expression domain to the CZ by preventing differentiation of the PZ (Reddy and Meyerowitz, 2005). Enlargement of SAM, as a reservoir of stem cells, was observed from embryonic stages in the *clavata* mutants (Clark et al., 1995; Schoof et al., 2000). The wild-type shoot meristem gives rise to rosette leaves and subsequently forms an inflorescence with numerous flowers. However *clavata* mutant exhibits an enlarged SAM phenotype throughout its lifecycle, and produces a fasciated inflorescence that initiates many flowers. The CZ of the floral meristem of *clv* mutants is also enlarged, resulting in an increased number of floral organs. CLV1 encodes an LRR-receptor-like kinase (LRR-RLK), and CLV2 encodes an LRR-receptor-like protein that lacks a kinase domain (Fig. 3a; Clark et al., 1997; Jeong et al., 1999). The CLV3 peptide, which encodes a 96-amino acid protein with a secretory signal peptide sequence in its N-terminal region and a CLE domain in its C-terminal region (Figs. 1 and 3a; Fletcher et al., 1999; Cock and McCormick, 2001), functions in Arabidopsis stem cells to restrict the size of the meristem.

The Arabidopsis *wus* mutant exhibits a SAM consumption phenotype. *WUS* encodes a homeobox transcription factor that is expressed in the organizing center of the SAM (blue region of the SAM in Fig. 3a). WUS has a central function in maintaining stem cell number and activity by providing a positive signal that maintains the undifferentiated state of the stem cells (Laux et al., 1996). Ectopic expression of *CLV3* or treatment with the CLV3 peptide induces the SAM consumption phenotype, which resembles that of *wus* mutants (Kondo et al., 2006; Strabala et al., 2006). Further, genetic studies revealed that *wus* is epistatic to *clv1*, *clv2*, *sol2/crn*, and *rpk2*, suggesting that WUS functions downstream of CLV3 receptors (Clark et al., 1995; Kayes and Clark, 1998; Kinoshita et al., 2010). Spatial expression analysis by mRNA *in situ* hybridization and RT-PCR revealed that the *WUS* expression level was up-regulated in mutants of the CLV3 receptors, indicating that CLV3 restricts the spatial expression of *WUS* to a region within the SAM organizing center (Kinoshita et al., 2010). The induction of *CLV3* repressed *WUS* expression in a period of just 3 hours (Müller et al., 2006). In turn, *CLV3* expression is positively regulated by *WUS*. This *CLV3*-*WUS* feedback system regulates the number of stem cells in the SAM (Schoof et al., 2000).

Mathematical models that explain autonomous pattern formation in the SAM have been proposed (Jönsson et al., 2005; Hohm et al., 2010). For example, the *WUS* expression domain is thought to be stabilized at the meristem center in the wild type and is disturbed in the *clv* mutants, and the expression domain is regenerated in the meristem center following CZ ablation. Fujita et al. (2011) extend the model to describe the mechanism underlying SAM proliferation and patterning by integrating information on cell divisions and spatial restrictions of the meristem into the reaction-diffusion dynamics that are based on the regulation of WUS by CLV.

The CLV3 peptides and corresponding receptors: complexity

Biochemical studies identified a mature form of CLV3 as a 12- or 13-amino acid peptide with two hydroxy prolines (RTVPhSGPhDPLHH(H)) (Fig. 1; Kondo et al., 2006; Ohyama et al., 2009). Chemically synthesized CLV3 is also functional in plants, and application of synthetic CLV3 peptides induced the SAM and RAM consumption phenotype, which resembles the CLV3 overexpression phenotype, suggesting that the synthetic CLV3 peptides function in the homeostasis of both the SAM and RAM (Kondo et al., 2006). Further, arabinosylation is observed at the hydroxyproline residues of the natural CLV3 peptide, and the activity of arabinosylated glycopeptides is much higher than that of synthetic peptides that lack sugar modification (Ohyama et al., 2009). The direct interaction between CLV3 and CLV1 was detected using synthetic or purified CLV3 peptides and membrane fractions of tobacco BY-2 cells expressing Arabidopsis *CLV1* (Ogawa et al., 2008). Post-translational arabinosylation of CLV3 was thought to increase the binding affinity of ligands for their receptors (Ogawa et al., 2008).

CLV1, CLV2, SOL2/CRN, and RPK2 function as CLV3 receptors (Fig. 3a and Table 2). *RPK2* encodes an LRR-RLK, and *SOL2/CRN* encodes a membrane-bound kinase that lacks an extracellular domain (Fig. 3a; Miwa et al., 2008; Müller et al., 2008; Kinoshita et al., 2010). CLV3 also binds to CLV2 physically in vitro, and CLV2 and CLV1 bind to the CLV3 with similar kinetics (Guo et al., 2010). A direct interaction between CLV3 and RPK2 remains to be demonstrated. Many combinations of receptors were proposed to exist based on different lines of evidence. Genetic and biochemical analyses mainly indicate that the following three major complexes perceive the CLV3 signal: CLV1, the CLV2-SOL2/CRN hetero-oligomer, and the RPK2 homo-oligomer (Zhu et al., 2009; Bleckmann et al., 2010; Kinoshita et al., 2010). However, biochemical studies revealed that SOL2/CRN can also function as a bridge, not only between CLV1 and CLV2, but also between CLV1 and RPK2, indicating the presence of large CLV receptor complexes composed of CLV1, CLV2, SOL2/CRN, and RPK2 (Zhu et al., 2009; Bleckmann et al., 2010; Betsuyaku et al., 2011). The composition of receptor complexes is not clearly affected by the CLV3 ligand (Zhu et al., 2009; Bleckmann et al., 2010). On

the other hand, the CLV3 ligand induces CLV1 phosphorylation (Betsuyaku et al., 2011). Functional differences between different types of CLV receptor complexes, and the function of CLV1 phosphorylation remain unknown. Genetic analysis showed that these three major receptor complexes are critical for the perception of CLV3 (Kinoshita et al., 2010). *clv1-101 clv2-101*, *clv1-101 rpk2-2,* and *clv2-101 rpk2-2* double mutants exhibited larger SAMs than any of the single mutants (Kinoshita et al., 2010). However, the SAM size of these double mutants was still smaller than that of the *clv3* single mutant (Kinoshita et al., 2010). The *clv1-101 clv2-101 rpk2-2* triple mutant showed not only a fasciated SAM, but also a massively overproliferated SAM, as seen in *clv3* single mutants (Kinoshita et al., 2010). This result indicates that the presence of these three receptor pathways is critical for perceiving the CLV3 signal precisely. Interestingly, SOL2/CRN lacks kinase activity under standard *in vitro* conditions. Further, the kinase-dead version, *sol2/crn* (K146E), can complement the abnormalities of the *crn-1* mutant. The finding that SOL2/CRN does not require kinase activity in the CLV3 signaling pathway further supports its role as a scafold (Nimchuk et al., 2011a).

CLV1 expression is restricted to the region surrounding the organizing center in the SAM, which is marked by *WUS* expression (Clark et al., 1997). *CLV2*, *SOL2/CRN*, and *RPK2* expression are widely observed in the SAM (Jeong et al., 1999; Bleckmann et al., 2010; Kinoshita et al., 2010). The distribution of these proteins in the SAM is also important for their function. In contrast to the uniform distribution of *RPK2* transcripts in the SAM, the fully functional protein product of *RPK2* expressed under the control of its own promoter and fused to a GFP tag was detected preferentially in the PZ and weakly in the CZ, where *CLV3* is expressed, suggesting that the *in situ* stability of RPK2 is highly regulated post-translationally (Kinoshita et al., 2010). The data further indicate that the other CLV receptors might also be regulated post-translationally in the SAM. Actually, CLV3 reduces the amount of plasma membrane-localized CLV1 by inducing CLV1 protein trafficking to lytic vacuoles (Nimchuk et al., 2011b). On the other hand, BAM1 and BAM2, two LRR-RLKs highly resembling to CLV1, bind to the CLV3-derived CLE peptide *in vitro*, and *BAM* overexpression compensates for the loss of CLV2 function *in vivo*, indicating that BAM1 and BAM2 function in the CLV3 signaling pathway (DeYoung et al., 2006; Guo et al., 2010).

Thus, the spatial localization of CLV receptors, i.e., CLV1, CLV2, SOL2/CRN, RPK2, BAM1, and BAM2, in the SAM is likely to be highly complex and still needs to be analyzed precisely. The specific spatial distribution of the CLV receptors might contribute to maintaining the SAM structure by limiting interacting combinations among them to exert certain specific activity in each domain of the SAM.

Downstream signaling components of CLV3

Genetic studies in Arabidopsis have shed light on the downstream signal components of CLV3 signaling pathway. The type-2C kinase-associated protein phosphatase (KAPP) interacts directly with and dephosphorylates the CLV1 kinase domain, and acts as a negative regulator of CLV signal transduction (Williams et al., 1997; Stone et al., 1998). The *poltergeist* (*pol*) mutant is indistinguishable from wild-type plants. However, a *pol* mutation suppresses the *clv1*, *clv2*, and *clv3* phenotypes (Yu et al., 2000). *POL* encodes a protein phosphatase type 2C (PP2C) protein with a predicted nuclear localization sequence, and the POL protein represents a unique subclass of plant PP2Cs (Yu et al., 2003). Mutation of the *POL* homolog, *POLTERGEIST LIKE 1* (*PLL1*), partially suppresses the phenotypes of *clv* mutants. A series of genetic studies demonstrated that POL and PLL1 are integral components of the CLV signaling pathway, and act downstream of CLV signaling to maintain *WUS* expression. POL and PLL1 directly bind to multiple lipids, and POL catalytic activity is up-regulated by phosphatidylinositol (4) phosphate (Gagne and Clark, 2010). In addition to phosphatases, the mitogen-activated protein (MAP) kinase cascade has been implicated in the phosphosignaling pathway that regulates *WUS* expression downstream of the CLV receptor-like kinases (Trotochaud et al., 1999; Torii, 2000; Clark, 2001; Jun et al., 2008; Butenko et al., 2009). Indeed, recent analysis of MPK activity in CLV signaling revealed that the exogenous application of CLV3 peptides stimulated MAP kinase 6 (MPK6) activity in wild-type Arabidopsis seedlings (Betsuyaku et al., 2011). Further, the MPK6 activity of *clv1* was about five times higher than that of the wild type (Betsuyaku et al., 2011). This indicates that CLV1 negatively regulates the MAP kinase cascades. Accordingly, conditional overexpression of a dominant negative form of *MAP kinase kinase 4, MKK4*, which is a known upstream kinase of MPK6, suppressed the abnormalities of *clv1* in the carpel (Betsuyaku et al., 2011). This is the first experimental evidence that demonstrates the possible involvement of the MAP kinase cascade in the CLV signaling pathway. An additional component of the CLV complex is a Rho GTPase-related protein (Rop) that is related to the Ras GTPase superfamily in animals (Trotochaud et al., 1999). Ras GTPases are typically associated with cytosolic mitogen activated protein kinase (MAPK) cascades (Downward, 2001), and the Rop may function with MKK4 and/or MPK6 in the CLV3 signaling pathway.

Intersection of the CLV signaling pathway and cytokinin

The CLV signaling pathway is partially regulated by the plant hormone, cytokinin. Localized perception of cytokinin establishes a spatial domain in which cell fate is respecified through induction of *WUS* expression (Leibfried et al., 2005). Cytokinin-induced *WUS* expression occurs through both CLV-dependent and -independent pathways. The expression of cytokinin-related genes is regulated downstream of *WUS*. *WUS* directly represses the transcription of several two-component *ARABIDOPSIS RESPONSE REGULATOR* genes (*ARR5, ARR6, ARR7*, and *ARR15* (Leibfried et al., 2005), which are known to act in the negative feedback loop that regulates cytokinin signaling (To et al., 2004). Feedback loops through cytokinin and CLV-WUS signaling control stem cell number in the SAM. Cytokinin biosynthesis and metabolism also regulate SAM homeostasis. The *lonely guy* (*log*) mutant in rice shows a remarkable reduction in SAM size (Kurakawa et al., 2007). LOG encodes an enzyme that directly converts inactive cytokinin nucleotides, such as iPRMP and tZRMP, into the bioactive freebase form. *LOG* is specifically expressed at the top of shoot meristems. Multiple steps in cytokinin metabolism and signaling are crucial for the maintenance of undifferentiated meristematic cells in the SAM.

Beyond Arabidopsis: Conservation of CLE functions in the SAM

The molecular relationship between the CLE ligand and LRR-RLK is conserved in various plant species. Maize *ESR* was firstly identified as a *CLE* gene (Opsahl-Ferstad et al., 1997). Rice *CLE* genes, *FLORAL ORGAN NUMBER2* (*FON2*; Suzaki et al., 2006), *FON2-LIKE CLE PROTEIN1* (*FCP1*; Suzaki et al., 2008), and *FON2 SPARE1* (*FOS1*; Suzaki et al., 2009), control the size of inflorescence and/or floral meristems. Mutations in *FON1* in rice cause enlargement of the floral meristem, resulting in an increased number of floral organs. *FON1* of rice encodes a receptor-like kinase with an LRR structure in the extracellular domain that is closely related to that of Arabidopsis *CLV1* (Suzaki et al., 2004). Likewise, in *Zea mays* (maize), *thick tassel dwarf1* (*td1*) encodes a CLV1-like receptor kinase (Bommert et al., 2005), and *fasciated ear2* (*fea2*), like *Arabidopsis CLV2*, encodes an LRR protein that lacks a cytoplasmic domain (Taguchi-Shiobara et al., 2001). Loss of function of these maize genes results in the enlargement of the inflorescence and floral meristems, which causes fasciation of the inflorescences and an increase in floral organ number.

ROLE OF CLE PEPTIDES IN MAINTAINING RAM HOMEOSTASIS

Arabidopsis stem cell niches in the RAM are well described at the anatomical level (Scheres et al., 2002). The root stem cell niche includes four cells that rarely divide and constitute the quiescent center, surrounded by four sets of initial cells that give rise to the different types of differentiated cells in the root (i.e., stele, cortex, endodermis, epidermis, lateral root cap, and columella cells) (Scheres et al., 2002). *CLV3* expression was not detected in the RAM. However, overexpression of *CLV3* or application of the synthetic CLV3 peptide induced a RAM consumption phenotype, which is similar to the effect of *CLV3* on the SAM (Strabala et al., 2006; Kinoshita et al., 2007). Many *CLE* genes and peptides, such as CLE19 and CLE40, had the same effects as CLV3 on the root when ectopically overexpressed or directly applied, respectively, and these effects were suppressed by *clv2*, *crn/sol2*, or *rpk2* mutations (Casamitjana-Martinez et al., 2003; Miwa et al., 2008; Müller et al., 2008; Kinoshita et al., 2010)). The observation that numerous *CLE* genes are expressed in the RAM, suggests that CLE signaling is responsible for RAM homeostasis in Arabidopsis (Jun et al., 2010). The roots of the *cle40* mutant grow in a wavy pattern (Hobe et al., 2003). The *cle40* mutant also exhibits a short root phenotype and irregular cell patterning in the RAM, indicating that CLE40 functions to maintain RAM homeostasis (Hobe et al., 2003). *WUSCHEL-related homeobox5* (*WOX5*) expression is restricted to QC cells in Arabidopsis (Haecker et al., 2004; Sarkar et al., 2007). A loss-of-function mutation in *WOX5* causes terminal differentiation, with enlarged QC cells and columella stem cells (Sarkar et al., 2007). Conversely, *WOX5* overexpression represses differentiation in the columella cells and results in the overproduction of columella initial cells (Sarkar et al., 2007). Promoter and gene swap experiments, such as *WUSp::WOX5* and *WOX5p::WUS* in the *wus* and *wox5* mutant, respectively, restored the mutant phenotypes (Sarkar et al., 2007). This result suggests that *WOX5* and *WUS* are interchangeable in stem cell control, and these *WOX* genes have similar functions in stem cell maintenance in the SAM and RAM (Sarkar et al., 2007). The CLV3 homolog, CLE40, and the WUS homolog, WOX5, seem to function in the RAM.

Stahl et al. (2009) demonstrated an interaction between CLE40 and WOX5. Increased CLE40 levels drastically alter the spatial expression pattern of *WOX5*, and promote stem cell differentiation (Fig. 3a and Table 2). The addition of synthetic CLE40 or CLV3 peptides promotes differentiation of columella stem cells into columella cells. However, CLE40 treatment had only minor effects on the *acr4* mutant. Similar to the *cle40* mutant, the *acr4* mutant had an expanded region of *WOX5* expression. *ACR4* encodes a receptor-like kinase protein that belongs to the CRINKLY4 family, in which receptor ectodomain is unrelated to the LRR domain found in CLV1, CLV2, and RPK2/TOAD2. ACR4 is thought to perceive CLE40 peptides and then repress *WOX5* expression (Stahl et al., 2009). In the RAM, a CLE40-WOX5 feedback loop, similar to that of the SAM CLV3-WUS feedback system, controls RAM homeostasis (Fig. 3a and Table 2). The use of unrelated receptor kinase opens the possibility of yet additional complexity in the biochemical basis of CLE peptide perception.

THE FUNCTION OF CLE PEPTIDES IN VASCULAR DEVELOPMENT

As we have introduced, some CLE peptides regulate the development of the SAM and RAM. CLEs function in another type of stem cell tissue called the vascular meristem, which consists of procambial cells (Fig. 3a and Table 2). The vascular tissues are important for providing mechanical support and distributing water and nutrients as well as other substances needed for growth and defense in plants. The plant vascular tissues form bundles that connect all parts of the plant and consist of one type of meristematic tissue, called procambial tissue, and two types of differentiated conductive tissues, named xylem and phloem (Fig. 3a and Table 2). The procambial cells are interspersed between phloem and xylem and are able to differentiate into both tissues. During vascular development, these procambial cells produce phloem cells on one side and xylem cells on the other. Recent studies showed that CLE peptides function in vascular development as signaling molecules and spatially and temporally regulate vascular development.

Tracheary Element Differentiation Inhibitory Factor (TDIF) / CLE41/CLE44 controls the fate of procambial cells in a non-cell-autonomous manner (Fig. 3a and Table 2; Ito et al., 2006; Hirakawa et al., 2010a). When *Zinnia* (*Zinnia elegans* L.) mesophyll cells are cultured in the presence of two hormones, auxin and cytokinin, the mesophyll cells transdifferentiate into tracheary elements (TEs; the main conductive cells of the xylem) (Fukuda, 1997). Differentiation of TEs is inhibited by extracellular factors in this xylogenic culture system. TDIF was isolated from some fraction of the conditioned medium. Analysis of the mass spectrometry data and amino acid sequencing showed that TDIF is a dodecapeptide with two hydroxyproline residues (Hyp), HEVHypSGHypNPISN (Ito et al., 2006). Chemically synthesized TDIF also inhibited TE differentiation. The amino acid sequence of TDIF is identical to that of Arabidopsis CLE41 and 44, and the TDIF is highly similar

to that of Arabidopsis CLE42 and 46. Treatment with most Arabidopsis CLE peptides affects Arabidopsis root meristem development; however, these peptides do not inhibit TE differentiation in *Zinnia*. In contrast, treatment with CLE41/44 and CLE42 do not affect root meristem development in Arabidopsis (Ito et al., 2006). Both the overexpression of CLE44 and exogenous treatment with TDIF promote procambial cell proliferation and inhibit xylem differentiation in Arabidopsis (Hirakawa et al., 2008). The CLE41/44 and CLE42 peptides are grouped together in the CLE peptide phylogenetic tree (Ito et al., 2006). These results suggest that TDIF has specific functions in vascular development.

In shoot meristem development, CLV3 is perceived by a leucine-rich repeat receptor-like kinase, CLV1 (Fig. 3a and Table 2; Clark et al., 1997; Ogawa et al., 2008). The mutants of genes that encode leucine-rich repeat proteins and are expressed in procambial cells were screened for TDIF sensitivity, because, by analogy to the CLV3-CLV1 signaling system, it is expected that some leucine-rich repeat receptor-like kinases function as receptors in procambial cells. T-DNA insertion lines of the *TDIF RECEPTOR* (*TDR*; At5g61480) gene were insensitive to TDIF treatment (Hirakawa et al., 2008). *TDR* is *PHLOEM INTERCALATED WITH XYLEM* (*PXY*) (Fisher and Turner, 2007). The *pxy* mutant was previously reported to show defects in the separation between phloem and xylem. Biochemical analysis showed that CLE41 directly binds to PXY/TDR; however, CLV3, CLE2, CLE9, and CLE19 peptides did not bind to PXY/TDR (Fig. 3a and Table 2, Hirakawa et al., 2008). The *pxy/tdr* mutant did not show any shoot meristem defects, which have been reported in *clv* mutants (Fisher and Turner, 2007; Hirakawa et al., 2008). Combined, these results establish PXY/TDR as a TDIF receptor.

How do TDIF and PXY/TDR regulate the development of vascular tissue? Genetic analysis indicates that CLE41/42 and PXY act in the same genetic pathway (Etchells and Turner, 2010). *CLE41* and *44* are expressed in the phloem and the adjacent pericycle (Hirakawa et al., 2008; Etchells and Turner, 2010). TDIF peptides were detected in the apoplasm surrounding two phloem precursor cells by immunohistochemical analysis (Hirakawa et al., 2008). However, *PXY/TDR* is expressed in procambial cells (Fisher and Turner, 2007; Hirakawa et al., 2008; Etchells and Turner, 2010). From these data, it has been suggested that TDIF, which is produced in the phloem, is perceived by PXY/TDR, promotes cell division, and inhibits the differentiation of xylem in procambial cells (Fig. 3a). The ectopic expression of *CLE41* using the *35S* promoter or xylem-specific promoters induces cell division in a different orientation to that observed using phloem-specific promoters (Etchells and Turner, 2010). When *CLE41* is expressed in the phloem, where *CLE41* is expressed endogenously, using the phloem-specific promoter *SUCROSE-PROTON SYMPORTER2* (*SUC2*) in a wild-type background, the orientation of cell division of procambial cells was similar to that in the wild type. Although *pSUC2::CLE41* lines have increased expression of *CLE41* compared with the wild type, this did not affect the orientation of cell division. In contrast, when *CLE41* is ectopically expressed by the 35S promoter or a xylem-specific promoter (i.e., *IRREGULAR XYLEM3*), the orientation of cell division is affected in these lines. However, overexpression of *CLE41* and *42* did not affect the orientation of cell division in the *pxy* mutant. These data showed that the orientation of cell division is determined by the direction in which TDIFs are distributed via PXY/TDR, but not by the concentration of TDIF.

TDIF regulates two developmental processes, namely the division of procambial cells and the differentiation of procambial cells into xylem (Fig. 3a). Recently, it has been shown that *WUSCHEL-related HOMEOBOX4* (*WOX4*) is a key target gene that is required for the division but not the differentiation of procambial cells into xylem in TDIF pathways (Fig. 3a; Hirakawa et al., 2010b). Expression of *pWOX4::GUS* is detected in the tissues closely associated with the vasculature in the whole plant. Patterns of *WOX4* expression are similar to *PXY/TDR* expression. TDIF induces *WOX4* gene transcription within 1 hour in a TDR-dependent fashion to regulate procambial cell division (Fig. 3a). Furthermore, a negative-feedback loop between CLE41 and PXY/TDR is important for determining the orientation of cell division (Etchells and Turner, 2010). Overexpression of *CLE41* negatively regulates the expression of the *PXY/TDR* gene. The transcript level of *PXY/TDR* expression was higher in *pxy* mutants (Fisher and Turner, 2007). Overexpression of *CLE41* and PXY/TDR dramatically increased the number of cells in the vascular bundle and interfascicular region (Etchells and Turner, 2010). These data suggest that receptor-ligand interactions between CLE41 and PXY/TDR determine the size of the vascular meristem and induce secondary growth in the interfascicular region (Fig. 3a).

Do other CLE peptides regulate vascular development? Exogenously applied CLE6, CLV3, and CLE19 have synergistic effects on vascular development when these peptides are treated with CLE41 or 42 peptides (Whitford et al., 2008). A single treatment with CLE6, CLV3, and CLE19 results in root meristem consumption but has no effect on vascular development. Binary treatment of each three peptides with CLE41 or 42 induced the growth of vascular tissue; however, these binary treatments did not enhance the effects on root mersistem function.

Group-A peptides, including CLE10, which was described by Hirakawa et al. (2011), inhibit protoxylem vessel formation in Arabidopsis roots by regulating cytokinin signaling via *ARR5* and *ARR6* (Hirakawa et al., 2011; Kondo et al., 2011b). Cytokinin plays an important role in vascular patterning and the differentiation of all cells except for protoxylem. Mutants of the genes related to cytokinin signaling and cytokinin receptors showed phenotypes such as increased protoxylem cell files and loss of other cell types in the root vasculature (Mähönen et al., 2000; Mähönen et al., 2006). Microarray analysis showed that treatment with CLE10 reduced the expression of *ARR5* and *ARR6* (Kondo et al., 2011b). The *arr5* and *arr6* mutants had defects in protoxylem formation. CLE10 was shown to suppress *ARR5* expression in a CLV2 dependent manner (Fig. 3a and Table 2; Kondo et al., 2011b). These data indicate that Group-A peptide treatment functions via CLV2 to activate cytokinin signaling and inhibit the differentiation of protoxylem by reducing the levels of negative regulators of cytokinin (Fig. 3a and Table 2).

CLE REGULATES PLANT SYMBIOTIC INTERACTIONS WITH MICROBES

CLE is active not only in endogenous developmental programs, such as meristem regulation and vascular development, but also in environmental responses, including biotic interactions with microbes (Fig. 3a and Table 2). Recently, legume nodulation, which enables the host plant to grow in nitrogen-poor soil, has been

reported to involve CLE, in regulating nodule formation (Kosslak and Bohlool, 1984; Okamoto et al., 2009; Mortier et al., 2010).

The leguminous plants are capable of establishing symbiosis with nitrogen-fixing soil bacteria through the formation of symbiotic organs called nodules. However, the number of nodules is strictly controlled by internal and external cues, as the excessive formation of nodules exacts a high metabolic cost on the host plant (Kosslak and Bohlool, 1984; Caetano-Anolles and Gresshoff, 1991). Soil nitrate has been reported to be a major environmental key in the regulation of nodule formation; thus, host plants can control the number of nitrogen-fixing organs by monitoring the external supply of nitrogen (Carroll et al., 1985). The internal regulation of nodulation is referred to as "autoregulation", and in this process earlier nodulation events suppress the subsequent formation of nodules via long-distant signaling between the root and the shoot (Caetano-Anolles and Gresshoff, 1991; Oka-Kira and Kawaguchi, 2006). The root-shoot communication consists of two long-distant signals; a root-derived signal generated in the nodulating root is perceived by the shoots and a subsequent shoot-derived signal restricts the number of nodules in the roots (Caetano-Anolles and Gresshoff, 1991; Oka-Kira and Kawaguchi, 2006).

The primary indication for the involvement of CLE in autoregulation was the isolation of autoregulation defective mutants, *har1*, *nts-1,* and *sunn*, which correspond to genes with high levels of sequence similarity to *CLV1* in *Lotus japonicus*, *Glycine max,* and *Medicago truncatula*, respectively. Grafting experiments indicated that HAR1, NTS-1, and SUNN function in the shoot to receive a root-derived signal and then to restrict the number of nodules in the roots (Delves et al., 1992; Krusell et al., 2002; Nishimura et al., 2002; Penmetsa et al., 2003). However, despite their high level of sequence similarity to CLV1, none of these mutants exhibited typical *clv* phenotypes in their SAMs (Oka-Kira and Kawaguchi, 2006). In addition, expression analysis revealed that the *HAR1* and *NTS* transcripts were not expressed in the SAM as strongly as in other parts of the shoot, such as leaves (Nishimura et al., 2002; Searle et al., 2003). It is possible that another *CLV1* homologue, *GmCLV1A*, might serve the role of *CLV1* in the SAM of *Glycine max* (soybean; Searle et al., 2003). Thus, duplication of *CLV1* might have resulted in differentiation of *NTS-1* and *GmCLV1A* functions. A similar evolutionary explanation has also been proposed for *HAR1*. Although the function of HAR1 and NTS-1 might have diverged from CLV1 activity to symbiotic nodule regulation, their high level of sequence similarities to CLV1 point to a potential conservation in the molecular machineries that underlie the CLV pathway and autonodulation signaling, further indicating that HAR1 and NTS-1 might receive CLE-like signal molecules derived from the infected roots.

This speculation was further supported by the recent finding that the *CLE* genes are involved in nodulation (Fig. 3b and Table 2; Okamoto et al., 2009; Mortier et al., 2010). The on-going genome project of *Lotus japonicus* revealed at least 39 genes containing the CLE domain, designated as *LjCLE* genes (Okamoto et al., 2009). Expression analysis identified three *LjCLE* genes, namely *LjCLE-RS1*, *LjCLE-RS2,* and *LjCLE3*, that were strongly and rapidly up-regulated in the roots upon rhizobial infection (Okamoto et al., 2009). Genetic analysis using *Agrobacterium*-mediated transformation of hairy roots with these three *LjCLE* genes revealed that *LjCLE-RS1* and *LjCLE-RS2*, but not *Lj*CLE3, regulated the number of nodules locally and systemically (Fig. 3b and Table 2; Okamoto et al., 2009). In addition, *LjCLE-RS1* and *LjCLE-RS2* were shown to suppress nodule formation systemically via the shoot in a *HAR1*-dependent manner, raising the possibility that these CLE proteins, presumably as mature peptides of 12 or 13 amino acids corresponding to the CLE domain, might function as root-derived mobile signals of nodule autoregulation (Fig. 3b and Table 2; Okamoto et al., 2009). However, it is still unclear if the mature products of *LjCLE-RS1* and *LjCLE-RS2* travel from the nodulating roots to the shoot (Fig. 3b). None of the CLE peptides, such as MCLV3 and TDIF, have been reported or considered to move such a long distance. The mature peptides produced from LjCLE-RS1 and LjCLE-RS2 might possess distinct molecular characteristics, for example, in the length of the peptide or in the post-translational modifications, to achieve long-distance transport from the roots to the shoot. Experimental evidence of translocation of these CLEs from the roots to the shoots and identification of their functional mature forms would further advance our understanding of autoregulation signaling.

The study of *LjCLEs* involved in autoregulation also provided a molecular explanation for the nitrate-dependent regulation of nodulation (Table 2). Mutations in *HAR1* and *NTS-1* have been known to show nitrate tolerance of nodulation, suggesting that nitrate-dependent signals are likely to be integrated into autoregulation signaling pathways (Carroll et al., 1985; Krusell et al., 2002). Okamoto et al. (2009) reported that *LjCLE-RS2* transcripts are strongly up-regulated in response to nitrate, which indicates that nitrate responsiveness of nodulation is mediated by *LjCLE-RS2*. Thus, among the CLEs in *L. japonicus*, *LjCLE-RS1* and *LjCLE-RS2* are responsible for autoregulation via *HAR1,* and *LjCLE-RS2* is also involved in nitrate-dependent regulation of nodulation in a *HAR1*-dependent manner (Fig. 3b and Table 2; Okamoto et al., 2009). During the course of evolution, leguminous plants might have integrated the nitrate-sensing system into autoregulation signaling using *LjCLE-RS2* to achieve developmental plasticity of nodulation in response to environmental conditions. It was recently reported that *LjCLE19* and *LjCLE20*, both of which appeared to serve as phosphate sensors in *L. japonicus*, might represent an additional example of the involvement of CLE in monitoring environmental conditions (Funayama-Noguchi et al., 2011).

The involvement of CLE in the autoregulation of nodulation is also observed in other legumes, such as *Medicago truncatula*, indicating an evolutionarily conserved CLE activity in nodule regulation of legumes (Table 2; Mortier et al., 2010). Amongst the 25 putative *CLE* genes in *M. truncatula*, *MtCLE12* and *MtCLE13* showed nodulation-related expression patterns and, similarly to *LjCLE-RS1* and *LjCLE-RS2*, were shown to regulate the number of nodules locally and systemically (Mortier et al., 2010). The predicted CLE peptide sequences of *MtCLE12* and *MtCLE13* are very close to those of *LjCLE-RS1* and *LjCLE-RS2*. *MtCLE13* promoter activity was observed in GUS expression assays in very young nodule primodia, three days after rhizobial inoculation, while the *MtCLE12* promoter was shown to be activated four days after inoculation (Mortier et al., 2010). GUS activity driven by both promoters was detected throughout the young nodule and, at later time points, was detected only in the apical tip of an elongated nodule (Mortier et al., 2010). This nodule-specific promoter activity of *MtCLE12* and *MtCLE13* is in striking contrast to the whole-root accumulation of *LjCLE-RS1* and *LjCLE-RS2* transcripts (Okamoto et al., 2009; Mortier et al., 2010). Interestingly,

MtCLE12 and *MtCLE13* exhibited an additional, systemic effect on petiole length and this effect by *MtCLE13* overexpression was suppressed by a mutation in a receptor-like kinase gene, *SUNN*, the closest homologue of *CLV1* in the *M. truncatula* genome (Table 2; Mortier et al., 2010). *SUNN* was previously shown to function in the autoregulation of nodules, similar to other legume *CLV1* homologues, *HAR1* and *NTS-1* (Penmetsa et al., 2003; Schnabel et al., 2005). However, the *sunn-1* mutation was shown to partially rescue the *MtCLE13*-dependent suppression of nodule formation, suggesting that other receptors might also be involved in the perception of MtCLE13 signals during the regulation of nodule number (Table 2; Mortier et al., 2010).

As described above, Arabidopsis CLEs can be divided into four groups, based on their activities (Table 1; Hirakawa et al., 2011). Intriguingly, the legume CLEs involved in the autoregulation of nodules show significant structural similarities to Arabidopsis CLE1-7 of the fourth group, which exhibits no known function to date. In analogy to the legume CLEs transmitting a long-distance signal from the roots to the shoot, one would expect this fourth group of CLE to function in an unknown long-distance signaling pathway from the roots to shoot.

In *L. japonicus*, another receptor-like kinase, *KLAVIER* (*KLV*), was demonstrated to function in the same genetic pathway as *HAR1* in the autoregulation of nodules, which provided us with a possible explanation for how the complex signaling pathways that control plant development and environmental responses are integrated (Fig. 3b and Table 2; Miyazawa et al., 2010). *KLV* was demonstrated to function in the shoot to mediate autoregulation signals in *L. japonicas* (Fig. 3b and Table 2). The *har1 klv* double mutant showed no additive nodulation phenotypes, and heterologously expressed HAR1 interacts with KLV in *N. benthamiana*, suggesting that these two proteins form a receptor complex for LjCLE in the same signaling pathway for nodule atuoregulation (Miyazawa et al., 2010). Intriguingly, a detailed phenotypic analysis of the *klv* mutant in the absence of symbiotic bacteria revealed that *KLV* functions not only in the autoregulation of nodules but also in the maintenance of the SAM and vascular development (Miyazawa et al., 2010). Fasciation, an enlarged SAM, and an increased number of floral organs in the *klv* mutant are hallmarks of the *clv*-related mutants (Miyazawa et al., 2010). Furthermore, the discontinuous vasculature of the *klv* mutant leaves resembled those of the *tdr* and *cle41* mutants (Miyazawa et al., 2010). These phenotypic features of the *klv* mutant strongly suggest that KLV might function in multiple pathways, such as the CLV and TDR pathways, in addition to autoregulation. KLV might perceive different CLEs in different tissues, and thereby activate the appropriate downstream signaling pathway. However, it is also plausible that KLV in the SAM might function, in addition to its roles in nodulation control and SAM maintenance, to integrate signals of autoregulation and environmental nitrogen sensing into the growth regulation of the SAM. A recent report demonstrated that *RPK2*, an Arabidopsis orthologue of *KLV*, functions as the third route of CLV signaling (Kinoshita et al., 2010). Whereas *rpk2* plants do not exhibit the *tdr* phenotype in terms of vasculature structure, the functions of RPK2 and KLV in the CLV pathway are clearly conserved. However, it is still unclear why Arabidopsis possesses three signaling routes to regulate the size of the SAM. One simple explanation is that these three pathways constitute a safety net to maintain the SAM, a crucial organ for plant growth and development. Alternatively, the three pathways may have overlapping activities and confer distinct and specific contributions to the regulation of the SAM. In support of this idea, at least the transcripts of the Arabidopsis receptors involved in three CLV pathways show different expression patterns in the SAM (Clark et al., 1997; Jeong et al., 1999; Müller et al., 2008; Kinoshita et al., 2010; Betsuyaku et al., 2011). This observation suggests that these receptors do not simply share the same function, but also possess unique activities. In this scenario, KLV and RPK2 might regulate SAM growth in response to CLV3 and environmental nutrient status, such as the availability of nitrate. Thus, legumes might have exploited such a long-distance signaling pathway for the autoregulation of nodule formation. The identification of *KLV*, which is responsible for the autoregulation of nodules and SAM maintenance, revealed the receptor for CLE signals as a potential integrator of environmental cues in an endogenous developmental signaling pathway. Future detailed studies of CLE and its receptor in SAM development and symbiosis will allow this possibility to be examined further.

CLE-LIKE GENES IN PLANT PARASITIC NEMATODES

We have reviewed accumulating evidence of the importance of CLE activity in shaping the plant in response to endogenous and environmental signals. However, recent studies also suggest that phytopathogenic microbes might have hijacked the CLE-mediated developmental signaling pathway of host plants, and that this might have improved the rate of colonization (Fig. 3a and Table 2).

Phytoparasitic nematodes are economically significant, as they can cause massive damage to many agriculturally important plant crops (Williamson and Hussey, 1996). To gain detailed insight into the establishment of nematode parasitic interactions with host plants, many efforts to identify “parasitic genes” required for parasitism of nematode have been carried out using different nematode species (Davis et al., 2000; Davis et al., 2008). In general, parasitic effecter proteins produced in the esophageal gland cells of nematodes were thought to be secreted from the nematode through its stylet into the plant tissue (Davis et al., 2000; Davis et al., 2008).

Attempts to target the secretory proteins from the esophageal gland cells of the soybean cyst nematode *Heterodera glycines* at the parasitic stage identified *HgCLE1* (formerly known as *2B10* and identical to *Hg-SYV46*) and *HgCLE2* (also known as *4G12*), both of which encode proteins that contain a C-terminal CLE domain (Wang et al., 2001; Gao et al., 2003; Wang et al., 2010). These two highly similar *HgCLE* genes encode proteins of almost 140 amino acids, and their N-terminal signal peptides and 12-amino acid CLE domains are identical (Wang et al., 2001; Gao et al., 2003; Wang et al., 2010). The specific antisera raised against the synthetic peptide corresponding to the CLE domain of these nematode proteins labeled the nematode effector secretion route from the dorsal lobe in the nematode (Wang et al., 2010). The antisera also detected the specific signal in the cytoplasm of a syncytial cell produced by the nematode, indicating that HgCLEs are delivered into the cytoplasm of a host plant cell through the stylet (Wang et al., 2010). Interestingly, complementation tests of the *clv3-1* plants using *HgCLE2* constitutively expressed under the control of the *CaMV35S* promoter resulted in the success-

ful complementation of the *clv* phenotype (Wang et al., 2005). Overexpression of *HgCLE2* in the Col-0 wild type resulted in the *wus*-like shoot phenotype and the short-root phenotype, both of which can be observed in *CLV3*-overexpressing plants (Wang et al., 2005; Davis, 2009). These data suggest that a nematode parasitic gene is able to mimic *CLV3* function *in planta*, and might be used to modify the host plant CLE signals (Olsen and Skriver, 2003; Wang et al., 2005; Davis et al., 2008; Mitchum et al., 2008).

Another CLE-like nematode gene, *16D10*, was isolated from the cDNA library derived from the esophageal gland cells of the root-knot nematode *Meloidogyne incognita* at the parasitic stage (Huang et al., 2006b). *16D10* encodes a short, 43-amino acid long protein that contains the predicted 30-amino acid signal peptide at the N-terminus (Huang et al., 2006b). The mature cleaved form of 16D10 is expected to occur as a 13-amino acid peptide, and share high sequence similarity to Arabidopsis CLE46, the function of which is still unknown (Huang et al., 2006b; Mitchum et al., 2008). Stable transgenic Arabidopsis plants overexpressing *16D10* without the predicted signal peptide exhibited accelerated growth of the root system, but no significant effect on shoot growth (Huang et al., 2006b). The phenotypes of Arabidopsis lines stably overexpressing full-length *16D10* were not reported because no transformants were obtained for unknown reasons, possibly due to strong toxic effects (Huang et al., 2006b). Thus, the cytosolic expression of *16D10* has an effect on plant growth, although plant CLE peptides,are thought to act on cell surface receptors in the intracellular space. Interpretation of 16D10 function as CLE mimicry by nematode needs further detailed experiments.

What are the functions of the nematode *CLE*-like genes? Do they contribute to phytoparasitic processes of the nematodes? Both cyst and root-knot nematodes have evolved sophisticated parasitic structures, syncytia and giant cells, respectively, which usually differentiate from the pericycle cells of the host plant roots, by modifying the host developmental programs in order to feed themselves (Fig. 3a; Williamson and Hussey, 1996; Davis et al., 2008; Mitchum et al., 2008). Successful colonization of the nematodes as endosymbionts inside the host root tissues results in high levels of reproduction, which represents the degree of pathogenicity. Knock-down of *16D10* expression by feeding the root-knot nematodes RNA interference (RNAi) constructs that target this gene before challenging the host roots resulted in a reduced egg formation rate in the roots (Huang et al., 2006a). Furthermore, transgenic Arabidopsis plants expressing the *16D10* RNAi construct had significantly lower levels of nematode reproduction. As ingestion of *16D10* dsRNA fragments by the nematode causes RNAi of the targeted genes, the dsRNA (siRNA) produced by plant tissue is most likely taken up by the nematode and results in *16D10* knock-down in the nematode during the infection process (Huang et al., 2006a) These data strongly suggest the importance of 16D10 as a phytoparasitic effecter of the root-knot nematodes. Accordingly, inoculation of Arabidopsis plants expressing the *Hg-SYV46* dsRNAi construct with the cyst nematodes was found to result in reduced reproduction rates of the nematodes in the host roots, indicating that *Hg-SYV46* is a key parasitic effector of the cyst nematodes (Patel et al., 2008). In turn, this trans-RNAi effect on parasitic *CLE*-like genes in phytopathogenic nematodes might be used to generate agricultural crops with broad resistance to the nematodes (Huang et al., 2006a).

Thus, *CLE*-like genes in the plant parasitic nematodes have crucial roles in the parasitic interaction with host plants. Recent studies on the nematode CLE-like proteins yielded further clues as to the molecular activities of these proteins during the parasitic interaction with plants. Yeast two-hybrid screening of a tomato cDNA library using 16D10 as bait isolated two *SCARECROW-like (SCL)* genes, the *AtSCL6* and *AtSCL21* homologues of tomato (Huang et al., 2006b). SCARECROW (SCR) and SHORT-ROOT (SHR), the founder members of the GRAS family proteins, regulate root radial patterning (DiLaurenzio et al., 1996; Helariutta et al., 2000). Similarly, most of the *AtSCL* genes are preferentially expressed in roots, suggesting their functional significance in root development (Pysh et al., 1999). Thus, AtSCL6 and AtSCL21 might possess some activity in root development (Huang et al., 2006b). The interaction between these two proteins and 16D10 was subsequently confirmed *in planta* using a transgenic Arabidopsis line overexpressing *16D10* (Huang et al., 2006b). Co-immunoprecipitation using a specific antiserum raised against 16D10 detected two interacting partners that had peptide signatures that corresponded to AtSCL6 and AtSCL21 by direct amino acid sequencing (Huang et al., 2006b). These data indicate that *16D10* may contribute to the formation of a root-knot by directly modifying the activity of these GRAS proteins early in the infection process. However, it is still unclear how 16D10 is processed and injected into the plant tissue. 16D10 may be produced in esophageal cells, secreted, and injected directly inside a plant cell through the stylet. In this scenario, the mature 16D10 peptide minus the putative signal peptide may interact with AtSCL transcription factors, as shown by Huang et al. (2006b). However, if this is the case, 16D10 does not mimic plant CLE activity, but operates via a different mechanism, since all plant CLEs are predicted to contain the secretion signal and to function in the apoplasm (Sawa, 2006). Importantly, overexpression of full-length *16D10* in Arabidopsis plants seems to be lethal, indicating that the predicted signal peptide of 16D10 may have a marked effect on the homeostasis of plant cells, which, in turn, might be important for the parasitic nematode (Sawa, 2006). Future studies of 16D10 are needed to address this question.

Recent immunolocalization studies using a specific antibody raised against the HgCLE peptide revealed that HgCLE was localized to the cytoplasm of a syncitium, which strongly suggests that the nematode delivers HgCLE directly into the plant cytoplasm via its stylet (Wang et al., 2010). However, deletion analysis of HgCLE2 prepropeptide domains, using the *CLV3* overexpression-like phenotype as a read-out when *HgCLE2* derivatives was overexpressed in Arabidopsis, demonstrated that the apoplastically-expressed nematode CLE domain is required, but not sufficient, for the induction of the *CLV3*-overexpression phenotype in Arabidopsis. In contrast, apoplastic activity of the CLE domain of Arabidopsis CLV3 is sufficient to trigger the *CLV3* overexpression-like phenotype (Kondo et al., 2006; Wang et al., 2010). Further detailed domain swap analysis among CLV3, HgCLE1, and HgCLE2 using the *CLV3*-overexpressing phenotype as a read-out showed that the variable domain of HgCLE2, which might represent a non-canonical secretion signal peptide, is required for the triggering of the *CLV3*-overexpression phenotype *in planta* (Wang et al., 2010). Moreover, a functional comparison of the variable domains (VDs) of HgCLE1 and HgCLE2 in Arabidopsis and soybean raised the possibility that the VDs might

confer host recognition specificity to the HgCLE prepropeptides (Wang et al., 2010). These data indicate that the nematode CLE-like proteins are primarily transmitted into the plant cytoplasm via the nematode's stylet and sequentially directed to the apoplasm by a non-conventional signal sequence in the VD. However, all the data are suggestive but not definitive yet to propose this novel function of the VD domain. Therefore, further analysis of the function of HgCLE VDs in host plants during nematode pathogenesis would be extremely valuable for assessing the ligand-mimic hypothesis of phytoparasitic nematodes.

One can test this hypothesis in another way, since HgCLE2 is shown to possess CLV3 activity when expressed in Arabidopsis (Wang et al., 2005). The predicted apoplastic CLE peptide derived from the HgCLE2 prepropeptide might be perceived by any of the known CLV3 receptors, namely, CLV1, CLV2, SOL2/CRN, and RPK2/TOAD2. One can simply test the effect of *HgCLE* overexpression in potential receptor mutants. Replogle et al. (2010) have tested this possibility and demonstrated that CLV2 and SOL2/CRN are required for the successful infection of the cyst nematode on the roots of Arabidopsis (Fig. 3a and Table 2). Promoter analysis of these two receptors revealed that they might be involved in the development of a syncitium induced by nematode infection (Fig. 3a and Table2; Replogle et al., 2010). This finding supports the hypothesis that the cyst nematodes hijack the plant innate CLE signaling pathway to reprogram the developmental status of the infected plant cells to induce the parasitic organ, the syncitium. Further functional analysis of the CLE peptides in plant root morphogenesis might give us a clue as to the control of agricultural damage caused by such phytopathogenic nematodes.

OTHER CLE FUNCTIONS IN PLANTS

Only three CLE-related pathways of ligand-receptor-downstream genes have been identified in Arabidopsis, and these include CLV3-CLV1-WUS in the SAM, CLE40-ACR4-WOX5 in the RAM, and CLE41-PXY/TDR-WOX4 in the vascular tissues (Fig. 3a). As mentioned previously, Arabidopsis has 32 *CLE* genes (Fig. 2). To elucidate the gene function of *CLE*, gain-of-function phenotypes of various *CLE* genes were analyzed in Arabidopsis. Overexpression of *CLV3* and the 17 *CLE* genes induced premature mortality and/or developmental timing delays in transgenic Arabidopsis plants (Table 3; Strabala et al., 2006). Overexpression of *CLV3* and 10 *CLE* genes resulted in growth inhibition of the SAM. Overexpression of nearly all of the *CLE* genes affected root growth. *CLE4* expression reversed the SAM proliferation phenotype of a *clv3* mutant to one of SAM arrest. The dwarf phenotype was also induced by the overexpression of five *CLE* genes. Overexpression of *CLE42* and *CLE44* resulted in distinctive shrub-like dwarf plants lacking in apical dominance (Table 3; Strabala et al., 2006). The ectopic expression of six *CLE* genes induced anthocyanin accumulation. The similarities among the overexpression phenotypes of many *CLE* genes correlate with similarities in their CLE domain sequences, suggesting that the CLE domain is responsible for the interaction with cognate receptors (Strabala et al., 2006).

It has been shown that almost all Arabidopsis tissues, including leaves, stems, various root tissues, branch points, all floral organs and the gynoceium except for stomata, express at least three *CLE* genes, by analysis of *CLE* promoter-driven reporters (Fig. 2, Tables 4 and 5; Sharma et al., 2003; Jun et al., 2010). Even highly specialized cell types, like trichomes and stipules,

Table 3. Summary of phenotypes resulting from CLE gene family member overexpression. Table modified from Strabala et al. (2006).

Gene (Background)[a]	Phenotype							
	wus-Like	Developmental Timing Delays[b]	Anthocyanin	Root Length[b]	Dwarf (14 DAG)[b]	Dwarf (21 DAG)[b]	>50% Mortality (Stage 5.10)[c]	Apical Dominance
CLV3	Y	Y	Y	Short	ND[d]	ND	67%	N
CLE2	Y	Y	N	Long	N	Y	N	N
CLE3	Y	Y	N	NC[e]	N	Y	N	N
CLE4	Y	Y	N	Long	ND	Y	N	N
CLE4 (Ler; clv3-2)	Y	Y	N	ND	N	Y	N	N
CLE5	Y	Y	N	Long	N	Y	N	N
CLE6	Y	Y	N	Long	N	Y	N	N
CLE7	Y	Y	N	Long	N	Y	N	N
CLE9	Y	Y	Y	Short	Y	Y	67%	N
CLE10	Y	Y	Y	Short	Y	Y	92%	N
CLE11	Y	Y	N	Short	Y	Y	92%	N
CLE13	Y	Y	Y	Short	Y	Y	96%	N
CLE18	N	Y	N	Long	N	N	N	Y
CLE19	N	Y	Y	Short	Y	Y	N	Y
CLE21	N	Y	Y	Short	Y	Y	N	Y
CLE25	N	Y	N	Long	N	N	N	Y
CLE26	N	Y	N	Long	N	N	N	Y
CLE42	N	Y	N	NC	Y	Y	N	N
CLE44	N	Y	N	NC	Y	Y	N	N

[a]Genetic background was Col-0 unless otherwise specified. [b]Relative to empty-vector control. [c]Only lines that showed >50% mortality prior to the appearance of the first floral bud (stage 5.10) are specified. [d]ND, Not done. [e]NC, No change.

Table 4. Summary of p*CLE*:*GUS* activity during vegetative development. Table modified from Jun et al. (2010).

CLE Gene	Shoot					Root				
	Shoot Apex[a]	Hypocotyl	Vasculature	Leaf Blade	Other[b]	Tip[c]	Vasculature	Ground Tissues	Epidermis	Other[d]
CLE1						+	+	+		
CLE2										+
CLE3					+		+	+		
CLE4					+		+			
CLE5					+		+			+
CLE6					+					+
CLE7							+	+		
CLE8										
CLE9					+					
CLE10					+	±[e]				
CLE11		+			+	+				+
CLE12		+	+				+			
CLE13						+				
CLE14									+	+
CLE16	+	+		+	+	+	+		+	+
CLE17	+	+		+	+	+	+	+	+	+
CLE18			+			+	+			
CLE20							+			+
CLE21		+			+					
CLE22		+	+			+	+			
CLE25			+			+	+			
CLE26		+	+			+	+			
CLE27	+	+		+		+				+

[a]Shoot apex includes SAM and rosette leaf primordia. [b]Other includes pith, stipules, stomata, hydathodes, leaf margins, trichomes, and the leaf base. [c]Root tip includes root cap, root apical meristem, and cell division zone. [d]Other includes root hair cells and lateral root branch points. [e]Eight of 15 p*CLE10*:*GUS* lines showed root tip expression.

Table 5. Summary of p*CLE*:G*US* activity during reproductive development. Table modified from Jun et al. (2010).

CLE Gene	Stem/ Pedicels	Branching Points	Cauline Leaves	Sepals/ Petals	Stamens		Gynoecium				Flower Base[a]
					Anthers	Filament	Stigma	Style	Ovary[b]	Ovules	
CLE1					+			+			
CLE2											
CLE3			+								
CLE4		+				+					+[c]
CLE5		+						+			+
CLE6		+				+					+
CLE7					+						
CLE8											
CLE9	+		+	+[d]							
CLE10		+	+				+	+	+	+	
CLE11					+			+			
CLE12	+[e]		+		+						+
CLE13	+[e]				+						+
CLE14				+[d]							
CLE16	+			+[e]		+			+		+
CLE17	+	+	+	+			+	+	+[c]		
CLE18			+	+[d,e]		+					
CLE20	+[e]										
CLE21		+							+		+
CLE22	+[e]		+	+[e]					+[e]		+
CLE25	+[e]				+				+[e]	+	
CLE26	+[e]		+	+[d,e]		+			+[e]		+
CLE27		+								+	+

[a]Includes receptacle and abscission zone. [b]Includes valves, replum, and septum. [c]Siliques only. [d]Sepals only. [e]Vasculature.

express *CLE* genes (Fig. 2). The expression patterns of many promoter-driven reporter lines overlaps, but is not identical. This result suggests that the CLE peptides have functions in relaying the precise positional cues locally. The expression of many *CLE* genes overlaps, and antagonistic and/or synergistic interactions might exist among CLEs.

The receptors of CLE peptides, CLV1, RPK2, ACR4, PXY/TDR, CLV2, and SOL2/CRN, have been characterized in Arabidopsis. Arabidopsis genomes have more than 200 *LRR-RLK* and more than 50 *LRR-RLP* (Shiu and Bleecker, 2001; Wagn et al., 2008). Thus, receptors and ligands are redundant. Overexpression of *CLE* genes that encode similar or the same mature peptide sequences has similar or the same activity. However, the endogenous expression patterns of these genes vary. This suggests that CLE peptides with similar or the same sequences could regulate different steps of plant development. Future analysis will reveal novel functions of CLE peptides

Recent study by Lee et al demonstrated that CLV3 is required not only for stem cell homeostasis but also for immunity in Arabidopsis (Lee et al, 2011). Stem cell-derived CLV3 signal was shown to be perceived by an LRR-RLK pattern recognition receptor for bacterial flagellin, FLAGELLIN INSENSITVE 2 (FLS2), to trigger a series of innate immune responses to limit the bacterial pathogen growth in the SAM (Lee et al, 2011). Surprisingly, CLV3-FLS2-mediated immunity was uncoupled from the known CLV3 receptor activities, such as CLV1 and CLV2, required for stem cell homeostasis (Lee et al, 2011). Using the benefit of encoding a great number of LRR-RLK receptors in the genomes, plants might have evolved an efficient strategy in which a CLE peptide signaling operates both in homeostasis and disease resistance of the SAM at the same time. Further dissection of CLE peptide activities in plant may uncover a similar complex CLE signaling by which plant achieves an organized and successful development during its life cycle.

CONCLUSION

In this chapter, we reviewed our current knowledge of the activity of CLE peptides in Arabidopsis and beyond. A number of detailed studies of *CLE* genes in Arabidopsis highlighted the functional significance of the CLE-receptor kinase-WOX module in regulating meristem activity, and this mechanism seems to be conserved among plant species. The existence of many CLEs and putative receptors might contribute to the fine-tuning of these key modules in various plant tissues. These well-organized signaling modules might have been adopted to control symbiosis effectively in the leguminous plants. On the other hand, the module might have been targeted by the phytopathogenic nematodes to modify and hijack the innate signaling pathway that regulates plant development. The finding that CLE function is required for plant-microbe interactions further emphasizes the importance of *CLE* genes in shaping plant morphogenesis. In addition to genetic and biochemical approaches, imaging of the spatial distribution of CLE peptides, as well as of the corresponding receptors, in plant tissues should advance our understanding of this finely-tuned CLE-mediated strategy of plant development.

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