CLE peptide ligands and their roles in establishing meristems

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Research in the past decade revealed that peptide ligands, also called peptide hormones, play a crucial role in intercellular communication and defense response in plants. Recent studies demonstrated that a family of plant-specific genes, *CLAVATA3* (CLV3)/*ENDOSPERM SURROUNDING REGION* (*ESR*) (*CLE*), which has at least 31 members in *Arabidopsis* genome, are able to generate extracellular peptides to regulate cell division and differentiation. A hydroxyl 12-amino acid peptide derived from the conserved CLE motif of CLV3 promotes cell differentiation, whereas another CLE-derived peptide suppresses the differentiation. These peptides probably interact with membrane-bound, leucine-rich repeat receptor-like kinases (LRR-RLKs) to execute the decision between cell proliferation and differentiation.

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Introduction

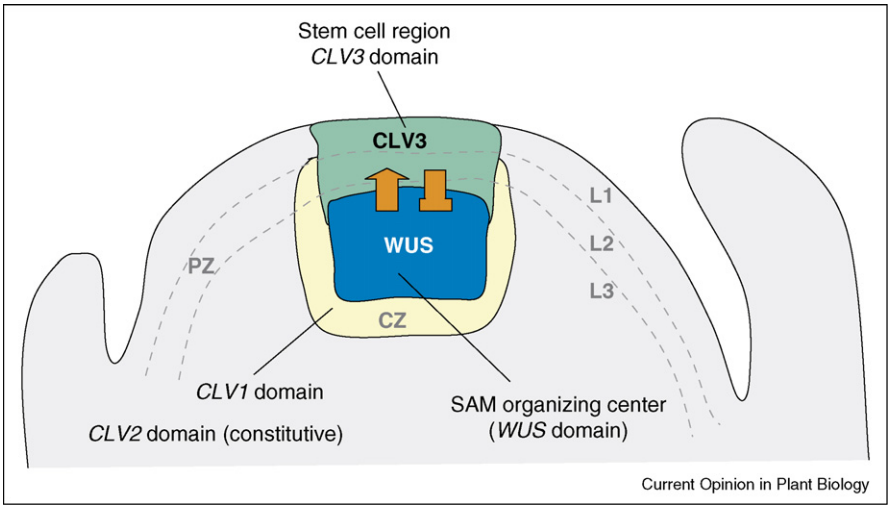
In a multicellular organism, individual cells need to sense positional signals from their surrounding cells, allowing them to coordinate their division and differentiation accordingly. This interaction allows cells in different tissues and organs to act in concert for growth and development. In animals, intercellular communication is largely mediated by small peptides and, to a lesser degree, by steroids [1]. In plants, most known intercellular communication is mediated by phytohormones, such as auxin, cytokinin, gibberellins, abscisic acid, ethylene, brassinosteroids, and jasmonic acid. These mobile secondary metabolites usually act as long-distance signals to allow cells to communicate in response to internal and external changes. In recent years, several secretory and non-secretory peptides, and in several cases their receptors, have been identified in plants [2,3••,4]. These peptides play important roles in mediating cell-to-cell communications in many biological processes; for example, systemin has a role in wounding responses, phytosulfokine plays a role in cell division, the *S*-locus cysteine rich (SCR) protein (synonym SP11) is central to anther–stigma interactions, and ENDO40 is involved in nodule development. The present review focuses on the exciting new developments in understanding the peptide signaling executed by CLAVATA3 (CLV3)/ ENDOSPERM SURROUNDING REGION (ESR)-related (CLE) proteins in *Arabidopsis*. The data reviewed in this article suggest that cells in the meristems use peptides as short-range signals to communicate with their neighboring cells and determine the fate of their progeny cells.

CLV3 restricts the number of stem cells in the shoot apical meristem

Over the past ten years, studies in *Arabidopsis* have provided concrete evidence showing that stem cells are indeed present in higher plants [5]. Stem cells that are positioned in the central zone (CZ) of the shoot apical meristem (SAM) are the source of totipotent cells that serve as the founders of all newly formed above-ground organs during the plant's life cycle [6]. The maintenance of a constant pool of stem cells requires tightly regulated machinery to sustain a dynamic equilibrium between cells in an undifferentiated state and cells that are destined to differentiate into various tissues and organs (Figure 1). At the molecular level, the balance is controlled by a feedback regulation loop, consisting of a WUSCHEL (WUS) homeobox transcription factor and a CLV3–CLV1–CLV2 (CLV) signaling pathway [7,8].

WUS, which is expressed in the L3 layer of the CZ in the SAM organizing center (OC), promotes the stem cell identity. Mutation of *WUS* leads to the differentiation of the stem cells, and consequently to the termination of the SAM; whereas overexpression of *WUS* induces ectopic stem cell formation. By contrast, the CLV signaling pathway limits the stem cell population by repressing *WUS* expression [7,8]. As such, mutations in any of the *CLV* genes (*CLV1*, *CLV2* or *CLV3*) lead to an enlarged vegetative and inflorescence meristem, and consequently, to an increased number of floral organs; whereas mutation of the *WUS* gene leads to a termination of the SAM. *CLV1*, which is expressed in the central L3 layer of the SAM, encodes a membrane-bound leucine-rich repeat receptor-like kinase (LRR-RLK) [9]. In the *Arabidopsis* genome, 223 LRR-RLKs have been identified, and ligands are unknown for most of these putative receptor kinases [10,11]. *CLV2* encodes an LRR-receptor-like

Figure 1



Structure of the SAM and the expression domains of several crucial SAM-related genes. *CLV1* is expressed in the L2 and L3 layers in the central zone (CZ), whereas *CLV3* is expressed in the L1, L2 and L3 layers of the same zone. *WUS* is expressed only in the L3 layer of the CZ. PZ, periphery zone.

protein (LRR-RLP) that is similar to CLV1 but lacks a kinase domain [12]. It has been suggested that CLV2 stabilizes CLV1 through dimerization via a disulfide bond and several proteins are involved in the complex [13], although the exact composition of the receptor complex is not known.

CLV3 encodes a putative extracellular protein that comprises 96 amino acids (AA) and is mainly expressed in the L1 and L2 layers of the CZ in the SAM, above the CLV1 domain [14]. Because of the similar phenotypes of *clv1*, *clv2* and *clv3* mutants, it is thought that CLV3 acts as a ligand for the CLV1–CLV2 receptor complex [14]. This hypothesis is supported by the discovery that *CLV3* encodes a secreted protein and that the secretion is essential for its function [15]. Because of the tight association of *CLV3* with stem cells in the SAM, *CLV3* has been considered to be a stem cell marker. Most likely, CLV3 peptides are secreted by the stem cells and then move downward to activate the CLV1 receptor kinase complex, which in turn suppresses the expression of *WUS* through an unknown signal cascade [16].

It is interesting to note the difference between animal and plant stem cells. The stem cells of animals are generally not able to regenerate [17], whereas stem cells in plants have a remarkable flexibility in regeneration. Laser ablation experiments have shown that, even when the entire CZ of the SAM is removed, cells in the periphery zone (PZ) are able to re-initiate the expression of *WUS* and to re-establish a new SAM OC and, consequently, the formation of stem cells [18]. Removal of the L1 layer of the SAM, however, leads to terminal differentiation of the SAM [18], suggesting that a stem-cell-restoring signal is provided by L1 cells.

With the data obtained to date, auxin is the most likely cue for the establishment of stem cells as it is transported from leaf primordia towards the summit of the meristem in the L1 layer [19]. Auxin might act as an upper stream signal to maintain the SAM OC, whereas the interaction between the WUS and CLV3–CLV1–CLV2 signal pathways maintains a continuous meristematic activity (a balance between cell proliferation and cell differentiation) in the SAM.

Dynamic function of *CLV3* in stem cell maintenance

Elegant experiments have been performed to monitor the direct effects of an increase in or depletion of *CLV3* in the SAM [20••,21••]. These were carried out by making use of inducible *CLV3* overexpression or of *CLV3* interference (*CLV3i*), combined with a continuous live observation of the SAM. These studies reveal new insights into the successive steps of CLV3 signaling in the SAM. Temporal induction of *CLV3* overexpression, and consequent downregulation of *WUS*, caused both decreased cell division in the CZ and a shift in the boundary between the CZ and the PZ, which resulted from the recruitment of cells from the CZ by organ primordia. The combination of these two processes resulted in a termination of the SAM [21••]. However, temporal expression of *CLV3i* caused an increase of *WUS* expression, followed by an increase in cell division and re-specification of the outer PZ cells as CZ cells, resulting in an enlargement of the SAM that is also seen in *clv* mutants [20••]. The overall conclusion of these studies is that *CLV3* functions not only in stem cell development but also in controlling the movement of cells from the CZ to the PZ and *vice versa*. Hence, *CLV3* is involved in setting the CZ/PZ boundary.

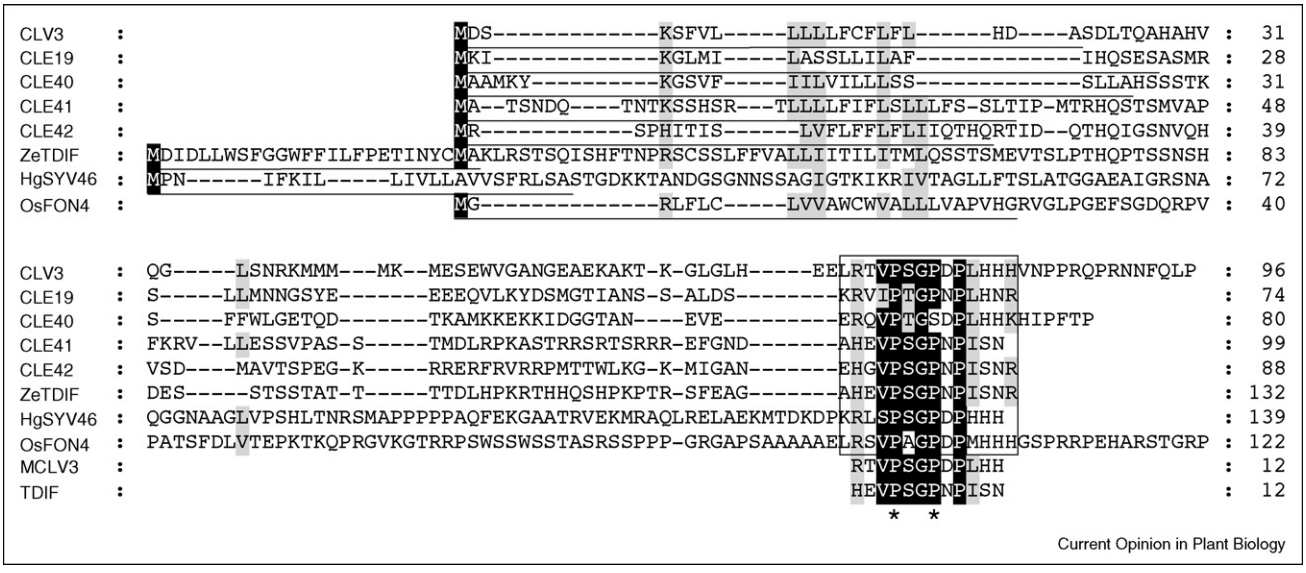
Molecular identity of CLE peptides

CLV3 belongs to the CLE family named after the first two founders: CLV3 from *Arabidopsis* and the ESR from maize [22]. All CLE proteins share common characteristics: they are small proteins (<15 kD) that have a putative secretion signal at their amino termini and a conserved 14-AA CLE motif at or near their carboxyl termini (Figure 2). In the *Arabidopsis* genome, *CLE* represents a family of at least 31 genes [22–26]. Among them *CLV3*, *CLE19* and *CLE40* have been studied extensively in recent years [23,25,27,28•]. Overexpression or root-specific expression of *CLE19* leads to a terminal differentiation of root meristem, implying a role for CLE19 in promoting cell differentiation or in inhibiting cell division [25,27]. The same phenotype was also observed when *CLV3* or *CLE40* was expressed under the control of *CaMV 35S* promoter [23]. Alignment of these proteins showed that they had very little similarity besides the CLE motif (Figure 2), which allowed Fiers *et al.* [28•] to speculate that the CLE motif might be the active domain of these proteins. To prove this hypothesis, synthetic peptides corresponding to the CLE motif of several CLE proteins were tested in a root assay [28•]. In this *in vitro* system, 14-AA peptides of CLV3, CLE19 and CLE40 (named CLV3p, CLE19p and CLE40p, respectively) were able to trigger the terminal differentiation of the root meristem, whereas peptides that had single amino acid changes or deletions were not functional, suggesting that the CLE motif is the functional cue [28•]. Interestingly, *clv2* did not respond to the peptide treatment, implying that CLV2 is functionally involved in the perception of these peptides in roots. Screening for mutations that can suppress the *CLE19* overexpression phenotype has led to the identification of two genetic loci, *SUPPRESSOR OF CLE19 1* (*SOL1*) and *SOL2*. Map-based cloning revealed that *SOL1* encodes a Zn-carboxylpeptidase. Although it has been proposed that this peptidase is involved in ligand processing, the exact function of SOL1 remains to be elucidated [27].

Further study using peptide assays showed that CLV3p, CLE19p and CLE40p are able to restrict the size of the SAM by restricting the *WUS* expression domain [29•]. Consistent with these results, domain-swapping and deletion analysis showed that the CLE motif of CLV3 functions independently of its adjacent flanking sequences [29•,30•]. More recently, the endogenous mature CLV3 peptide (MCLV3) was identified by *in situ* matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analyses of transgenic callus carrying the *CaMV35S*::*CLV3* construct. MCLV3 (RTVP^{h}SGP^{h}DPLHH) contains 12 AA, with hydroxyl groups attached to two of the three proline residues [31••]. Although hydroxylation is not required for peptide signaling by MCLV3, it might enhance the stability of the peptide.

Recently, a rice mutant that displayed enlarged shoot and floral meristems was shown to be mutated in a gene that is homologous to *CLV3* [32]. This gene was named *FLORAL ORGAN NUMBER4* (*FON4*). Interestingly, exogenous applications of both FON4p, which corresponds to the 14-AA CLE motif of FON4, and CLV3p resulted in a termination of the SAM in rice; and treatment of CLV3p

Figure 2



Alignment of several CLE proteins and the mature peptides. Sequences that are underlined are signal sequences, whereas the framed sequences are the CLE motif. Note that these peptides have very little similarity except the conserved carboxy-terminal CLE motif. MCLV3 and TDIF represent mature peptides, in which the first and the second proline (indicated by asterisks) are hydroxylated.

in rice also leads to consumption of root meristems, suggesting that the CLV pathway is relatively conserved between dicots and monocots.

Dual function for CLE peptides?

A CLE-like peptide, designated Treachery Element Differentiation Inhibitory Factor (TDIF), has been isolated from *Zinnia elegans* mesophyll cell culture as a suppressor of xylem development [33•]. This 12-AA peptide (HEVP^{h}SGP^{h}NPISN) with hydroxylated prolines, which is similar to MCLV3 [31••], is able to promote cell division and to suppress treachery element differentiation at a concentration of 10^{-11}M [33•]. TDIF is identical to CLE41 and CLE44 in the CLE motif. Examination of CLE peptides from *Arabidopsis* in *Zinnia* cultures showed that peptides derived from CLE41/CLE44 and CLE42 were able to suppress xylem differentiation. This is in contrast to CLV3, which promotes xylem differentiation [33•]. It is interesting, in this context, that overexpression of *CLE19* in *Arabidopsis* results in a failure in the connection of the xylem network, among other phenotypes [25]. Extensive vascular islands have been observed in the transgenic line carrying the *CaMV35S*::*CLE19* construct. The question of why and how some CLE peptides promote cell differentiation whereas others suppress cell differentiation remains to be answered.

CLE peptides outside the plant kingdom

The only known *CLE* gene outside of the plant kingdom is *HgSYV46* from the parasitic soybean nematode *Heterodera glycines* [34•,35]. The oesophageal gland cells of nematodes actively synthesize secretions that are injected through the stylet (i.e. oral spear) into plant cells to change the cell identity to that of specific feeding cells [36]. *HgSYV46* is specifically expressed in the dorsal oesophageal gland cells, and it encodes a protein that contains a putative signal sequence at its amino terminus and a CLE domain near its carboxyl terminus [34•,35]. When *HgSYV46* is expressed in *Arabidopsis* under the control of a *CaMV35S* promoter, it is able to complement the *clv3-1* mutant. In a wildtype background, overexpression caused termination of the shoot and root meristem, similar to *CLV3* and *CLE40* overexpression [34•]. The exact origin and function of the HgSYV46 is still unknown, but the gene might have been adapted from plants through horizontal gene transfer and might imitate the function of an endogenous CLE peptide to promote the differentiation of root cells into specific feeding cells.

Conclusions and future prospects

The plant-specific *CLE* genes, with at least 31 members in the *Arabidopsis* genome, might have introduced us to a whole new class of peptide ligands of higher plants. They appear to function in short-range cell-to-cell communication to coordinate the decision between cell proliferation and cell differentiation in meristems. The even larger family of LRR-RLKs could be major receptors that are involved in the perception of these peptide signals. Studies of CLV3, CLE19, CLE40, TDIF and FON4, carried out in the past few years, have provided several important technological tools for the functional analyses of these genes and peptides. Questions that remain to be answered include: what are the functions of each individual CLE and with which receptor(s) do they interact? How are the processing and modification of peptides regulated? And what kind of downstream signaling pathways are the peptides engaged in? By answering these questions, we might enter a new era in understanding how plant cells communicate with their neighbors.

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