Cell. Mol. Life Sci. 65 (2008) 743 – 755
1420-682X/08/050743-13
DOI 10.1007/s00018-007-7411-5

Cellular and Molecular Life Sciences

Review

The CLE family of plant polypeptide signaling molecules

J. H. Jun[a,b,†], E. Fiume[a,b,†] and J. C. Fletcher[a,b,*]

[a] Plant Gene Expression Center, USDA/UC Berkeley, 800 Buchanan Street, Albany, California 94710 (USA)
[b] Department of Plant and Microbial Biology, University of California, Berkeley, California 94720 (USA),
Fax: +1 510 559 5678, e-mail: fletcher@nature.berkeley.edu

Received 6 September 2007; received after revision 13 October 2007; accepted 23 October 2007
Online First 24 November 2007

Abstract. Polypeptide ligands have long been recognized as primary signaling molecules in diverse physiological processes in animal systems. Recent studies in plants have provided major breakthroughs with the discovery that small polypeptides are also involved in many plant biological processes, indicating that the use of polypeptides as signaling molecules in cell-to-cell communication is evolutionarily conserved. The CLAVATA3 (CLV3)/ENDOSPERM SURROUNDING REGION (ESR)-related (CLE) proteins are currently the best understood family of small polypeptides in plants. The recent isolation of MCLV3 from *Arabidopsis* and TDIF from a *Zinnia* cell culture system indicates that biologically active CLE polypeptides are produced by post-translational proteolysis and modification, similar to peptide hormone production in animals and yeast. Here, we review exciting discoveries involving the identification of the CLE proteins and their functions in various aspects of plant development, including restriction of stem cell accumulation by CLV3 and inhibition of xylem differentiation by TDIF.

Keywords. Polypeptide ligand, *Arabidopsis*, signaling, CLAVATA3 (CLV3)/ENDOSPERM SURROUNDING REGION (ESR)-related (CLE), receptor-like kinase, meristem, xylem differentiation.

Introduction

Cells in multicellular organisms must communicate with one another in order to regulate their growth and division and to coordinate their functions. Since the discovery of insulin in 1922 [1] established the fact that polypeptides can be used as signaling molecules in a diverse range of physiological processes, extracellular peptide ligands have been found to be key mediators of cell-to-cell communication in animal systems [2, 3]. For example, the transforming growth factor-beta (TGF-β) and epidermal growth factor (EGF) superfamilies consist of diverse groups of polypeptide growth factors that regulate cell duplication, migration, differentiation and survival [4, 5]. One feature of these polypeptide signals is that they are produced from larger pre-pro-proteins that undergo proteolytic cleavage of the signal peptide and the pro-domain before displaying biological activity [6]. The active ligands are then perceived at the cell surface, most commonly by transmembrane receptor protein-tyrosine kinases (PTKs). Although each growth factor induces a distinct downstream signaling pathway, the common theme of receptor kinase signaling is that binding of the ligand to the receptor converts the latter into an active state, initiating a cascade of intracellular protein phosphorylation that transduces the signal inside the cell. The signal is propagated through intracellular mediators such as MAP kinases, tyrosine phosphatases and Ras proteins, ultimately targeting one or more nuclear transcription factors and leading

† These authors contributed equally to this work.
* Corresponding author.

to an output that alters the gene expression program of the cell [2, 7].

In contrast to these well-understood animal polypeptide signaling systems, our historical understanding of intercellular signaling in plants has largely stemmed from the characterization of relatively simple lypophilic compounds called phytohormones, which include auxin and cytokinin [8]. Plant hormones play a crucial role in controlling the way in which plants grow and develop and mediate their physiological responses to environmental stimuli. Thus, for many years, phytohormones have been considered to be the major plant signaling molecules.

However, since 1991 [9] when systemin was discovered as the first functional plant polypeptide, many secretory and non-secretory polypeptides in plants have been recognized as being involved in many biological processes. Systemin, an 18-amino-acid polypeptide derived from a larger precursor called prosystemin, was isolated by biochemical purification based on its proteinase inhibitor-inducing activity in tomato and plays a role in wounding responses [10]. A number of other families of plant polypeptides have subsequently been identified [11], including several with members that play roles in growth and development. Phytosulfokine (PSK) is a 5-amino-acid peptide with a sulfated tyrosine residue that regulates cellular dedifferentiation and redifferentiation in cooperation with auxin and cytokinin [12]. POLARIS (PLS) appears to be required to maintain responsiveness to exogenous auxin and cytokinin during *Arabidopsis* root and vein development [13]. INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) is involved in *Arabidopsis* floral abscission [14], whereas the related ROTUNDIFOLIA4 (ROT4) and DEVIL1 (DVL1) polypeptides appear to control polar cell proliferation [15]. Both IDA and ROT4/DVL1 are members of multi-gene families in *Arabidopsis* [14, 15].

The *CLE* family of plant-specific genes, named after its founding members *CLAVATA3* (*CLV3*) from *Arabidopsis* [16] and *EMBRYO SURROUNDING REGION* (*ESR*) from maize [17], encode one of the largest families of plant polypeptides identified to date [18, 19]. The *Arabidopsis* genome contains 32 *CLE* genes, and CLE family members are present in many other plant species including rice, alfalfa and tomato [18, 20]. The *CLV3* gene was isolated by genetic screening in *Arabidopsis* [21] and plays a key role in regulating plant growth and development [16, 21]. Although no functional information from mutant analysis is available for the vast majority of *CLE* genes, several CLE proteins act in different aspects of plant development. This review provides an overview of the CLE polypeptides, their activities and signal transduction systems, including potential receptors and downstream target genes.

Molecular characteristics of *CLE* gene family members

Members of the CLE family of signaling molecules share several common molecular characteristics [18] (Fig. 1). First, they encode small polypeptides of less than 15 kDa in molecular mass. Second, they contain a short stretch of hydrophobic amino acids at their amino termini that can target the polypeptide to the secretory pathway. Third, although at the DNA and protein level their sequences are largely unrelated, they share a conserved stretch of 14 amino acids, known as the CLE domain (Table 1), which is located close to or at the carboxyl terminus. Finally, despite their poor overall sequence conservation, all members of the family share certain structural parameters such as charge, hydrophilicity and length.

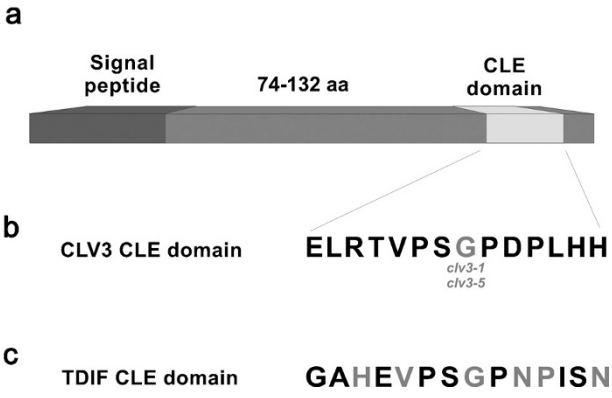

Figure 1. Molecular characteristics of the CLE proteins. (*a*) Schematic view of a generic CLE protein. (*b*) Amino acid sequence of the *Arabidopsis* CLV3 CLE domain. The natural product of the *CLV3* gene is a dodecapeptide corresponding to the central 12 amino acids of the CLE domain. Shown in light gray is the highly conserved glycine residue that is mutated in two *clv3* alleles. (*c*) Amino acid sequence of the *Zinnia* TDIF CLE domain. The natural product of the *TDIF* gene is a dodecapeptide corresponding to the last 12 amino acids of the CLE domain. Shown in light gray are amino acids known to be crucial for TDIF peptide function.

The 32 *Arabidopsis CLE* genes are scattered across the genome, with loci on each of the five chromosomes. Their fairly large number is most likely the result of whole-genome duplications and reshufflings, which are known to have happened at least three times in *Arabidopsis* evolution [22], as well as localized gene duplications followed by gene retention. The hypothesis that *CLE* gene number has risen at least in part thanks to localized gene duplication is supported by

Table 1. The CLE polypeptide family.

CLE	Sequence of dodecapeptide	AGI/ Accession No.	Size (aa)	Biological function				References
				Loss-of-function	Gain-of-function			
					SAM	RAM	TE	
CLV3	RTVPSGPDPLHH	At2g27250	96	shoot meristem	+	+	–	[52, 19, 41, 54, 56]
CLE1	RLSPGGPDPRHH	At1g73165	74	unknown	n.d.	–	–	[52, 54]
CLE2	RLSPGGPDPQHH	At4g18510	75	unknown	+	–	–	[52, 19]
CLE3	RLSPGGPDPRHH	At1g06225	83	unknown	+	–	–	[52, 19]
CLE4	RLSPGGPDPRHH	At2g31081	80	unknown	+	–	–	[52, 19]
CLE5	RVSPGGPDPQHH	At2g31083	81	unknown	+	–	–	[52, 19, 56]
CLE6	RVSPGGPDPQHH	At2g31085	81	unknown	+	–	–	[52, 19, 54]
CLE7	RFSPGGPDPQHH	At2g31082	86	unknown	+	–	–	[52, 19]
CLE8	RRVPTGPNPLHH	At1g67775	86	unknown	n.d.	+	–	[52, 54]
CLE9	RLVPSGPNPLHN	At1g26600	120	unknown	+	+	–	[52, 19, 54]
CLE10	RLVPSGPNPLHN	At1g69320	107	unknown	+	+	–	[52, 19]
CLE11	RVVPSGPNPLHH	At1g49005	99	unknown	+	+	–	[52, 19, 54]
CLE12	RRVPSGPNPLHH	At1g68795	118	unknown	n.d.	+	–	[52, 54]
CLE13	RLVPSGPNPLHH	At1g73965	107	unknown	+	+	–	[52, 19, 54]
CLE14	RLVPKGPNPLHN	At1g63245	80	unknown	n.d.	+	–	[52, 54]
CLE16	RLVHTGPNPLHN	At2g01505	103	unknown	n.d.	+	–	[52]
CLE17	RVVHTGPNPLHN	At1g70895	99	unknown	n.d.	+	–	[52]
CLE18	RQIPTGPDPLHN	At1g66145	101	unknown	–	+	–	[52, 19]
CLE19	RVIPTGPNPLHN	At3g24225	74	unknown	–	+	–	[52, 53, 19, 54, 56]
CLE20	RKVKTGSNPLHN	At1g05065	83	unknown	n.d.	+	–	[52]
CLE21	RSIPTGPNPLHN	At5g64800	106	unknown	–	+	–	[52, 19, 54]
CLE22	RRVFTGPNPLHN	At5g12235	103	unknown	n.d.	+	–	[52, 54]
CLE25	RKVPNGPDPIHN	At3g28455	81	unknown	–	+	–	[52, 19, 54]
CLE26	RKVPRGPDPIHN	At1g69970	118	unknown	–	+	–	[52, 19, 54]
CLE27	RIVPSCPDPLHN	At3g25905	91	unknown	n.d.	+	–	[52]
CLE40	RQVPTGSDPLHH	At5g12990	80	root	+	+	–	[52, 51, 56]
CLE41	HEVPSGPNPISN	At3g24770	99	unknown	n.d.	–	+	[52]
CLE42	HGVPSGPNPISN	At2g34925	88	unknown	–	–	+	[52, 19]
CLE43	RRIPSSPDRLHN	At1g25425	96	unknown	n.d.	n.d.	n.d.	[19]
CLE44	HEVPSGPNPISN	At4g13195	112	unknown	–	–	+	[52, 19]
CLE45	RRVRRGSDPIHN	At1g69588	124	unknown	n.d.	+	–	[52]
CLE46	HKHPSGPNPTGN	At5g59305	76	unknown	n.d.	–	–	[52]
OsFON4/ 2	RSVPAGPDPMHH	BAF41977	122	shoot meristem	+	–	n.d.	[20, 46]
ZeTIDF	HEVPSGPNPISN	ABL67522	132	unknown	n.d.	n.d.	+	[52]
HgSYV46	RLSPSGPDPHHH	AF273728	139	unknown	+	+	n.d.	[63]

Alignment of the CLE dodecapeptides was performed using MUSCLE [70]. Each color represents a different amino acid residue. Gain-of-function phenotypes were observed in either *Arabidopsis* transgenic *CLE* over-expressing lines or plants treated with synthetic CLE peptides. SAM (shoot apical meristem) or RAM (root apical meristem) termination is denoted by +, whereas – indicates no meristem termination phenotype. The suppression of tracheary element (TE) differentiation is denoted by +, whereas – indicates a TE differentiation phenotype. n.d., not determined.

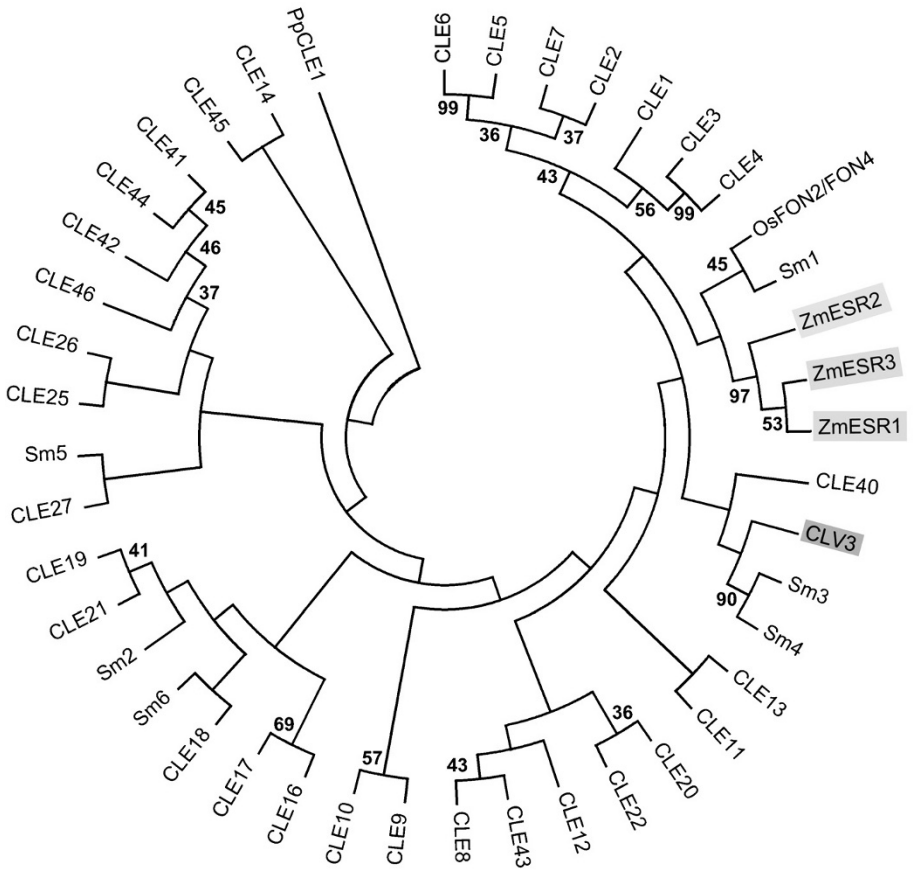

Figure 2. CLE protein phylogeny. The evolutionary history was inferred using the neighbor-joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. Multiple-sequence alignment of CLE proteins was performed using MUSCLE [70]. Percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) higher than 35 % are shown next to the branches. Phylogenetic analyses were conducted using MEGA4 software [71].

the presence of a tight cluster of highly similar *CLE* genes (*CLE4/5/6/7*) on chromosome 2 and by a looser clustering of *CLE* genes among repetitive sequences at the bottom of chromosome 1. In particular the presence of the *CLE4/5/6/7* cluster, which is a clear example of tandem repeats, is a sign of relatively recent gene duplication and suggests that these *CLE* genes may still be functionally redundant.

Phylogenetic analysis of the CLE proteins is challenging because of their low overall conservation and short length. As a result, trees are not very well supported, as illustrated by a phylogeny of the known plant CLE proteins in Figure 2. Nonetheless, some branches are supported by high bootstrap values and unveil putative couples of *Arabidopsis* paralogs, including CLE3 and CLE4, CLE5 and CLE6, CLE9 and CLE10, and CLE16 and CLE17. The phylogenetic analysis also suggests that CLE1 through CLE7 constitute a group of related proteins, as do CLE41, CLE42, CLE44 and CLE46.

CLE gene expression analysis provides valuable information for understanding the evolutionary forces shaping the family and the functions of the individual members. RT-PCR experiments performed with most of the *Arabidopsis CLE* genes [23] reveal that many family members have a broad range of expression, whereas a few show more restricted distribution. All *Arabidopsis* tissues tested to date express multiple *CLE* genes and the overlap among different *CLE* gene expression patterns is conspicuous: most tissues analyzed express many members at the same time. Pollen is the tissue that expresses the lowest number of *CLE* genes, with only six represented. However, RT-PCR data only provide a rough picture of the expression domains. High-resolution spatial expression patterns of *CLE* mRNAs in tissues and group of cells obtained by RNA *in situ* hybridization and/or promoter-reporter fusions will give a much more precise idea of the extent of expression overlap and possible functional redundancy.

Taking into consideration the limits of RT-PCR data, it is nonetheless interesting to note that close relationships among *CLE* genes at the evolutionary level are not always coupled with shared expression patterns. For example, although *CLE3* and *CLE4* seem to be clear paralogs on the basis of phylogenetic analysis (Fig. 2), their expression patterns do not completely overlap, suggesting that sub-functionalization may have occurred mainly through cis-acting mutations in their regulatory sequences. Conversely, the putative paralogs *CLE5* and *CLE6* share the same expression patterns and are likely functionally redundant.

Biological functions of the *CLE* genes

CLV3

CLV3 plays a critical role in the maintenance of stem cell reservoirs in *Arabidopsis* shoot apical meristems (SAMs). Whereas most animal organs are formed during embryogenesis, higher plants develop post-embryonically from shoot and root apical meristems at their growing tips. These meristems contain niches that promote stem cell survival and the production of descendants destined for differentiation into lateral organs such as leaves and flowers. In order to maintain a functional SAM throughout development, coordination between the loss of stem cells via differentiation and their replacement through cell division must be precise [24]. Insight into the regulation of normal SAM function has come from genetic studies in *Arabidopsis*. Loss-of-function mutations in *CLV3* cause excess stem cell accumulation in shoot apical and floral meristems [21]. Since the SAM of a mature *clv3* embryo is already enlarged compared to that of a wild-type embryo, *CLV3* function is required from an early developmental stage. The SAM size increase becomes more dramatic with time and results in the formation of a strap-like, fasciated stem and the production of extra flowers and floral organs, indicating that *CLV3* acts to restrict stem cell proliferation throughout development [21].

The *CLV3* gene encodes a small 96-amino-acid polypeptide with a predicted 18-amino-acid secretion signal at the amino terminus [16]. *CLV3* mRNA is expressed in the surface cell layers of the central zone (CZ) [16], which lie at the apex of the SAM and act as a reservoir of stem cells that replenish the peripheral zone (PZ) progenitor cells that populate lateral organ primordia. Genetic and immunological studies showed that CLV3 protein is transported through the secretory pathway and that secretion into the extracellular space is required for its normal function [25]. 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 [26]. MCLV3 consists of a 12-amino-acid sequence (RTVP^{h}SGP^{h}DPLHH) generated from near the C-terminal end of CLV3, indicating that the biologically active CLV3 polypeptide is produced by post-translational proteolysis, as are peptide hormones in animals and yeast [27].

clv1 and *clv2* null mutants have similar but weaker phenotypes than *clv3* null mutants [28, 29], and epistasis analysis strongly indicates that the three *CLV* genes act in the same pathway to maintain SAM activity by restricting stem cell accumulation. *CLV1* encodes a receptor-like serine/threonine kinase that contains 21 leucine-rich repeats in the extracellular domain [30]. *CLV2* encodes a receptor-like protein that is similar to CLV1 but lacks a kinase domain [31], and data suggest that CLV2 forms a disulfide-linked heterodimer with CLV1 [32]. Although biochemical studies indicate that the CLV1, CLV2 and CLV3 proteins form an active receptor complex [32], the model of direct ligand-receptor interaction remains to be confirmed. *CLV1* is expressed in a small group of cells just beneath the *CLV3* expression domain [30] and *CLV2* expression is also detected in shoots and flowers [31], suggesting that CLV3 function might be mediated by activation of a CLV1/CLV2 receptor complex in neighboring cells. However, the demonstration that *clv1* null alleles confer weak phenotypes and strong *clv1* alleles have dominant negative activity suggests that other receptor-like kinases (RLKs) have overlapping functions with CLV1 to regulate shoot apical meristem function [33].

Several other intracellular pathway components have been identified that modulate CLV signaling (Fig. 3). 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 [34, 35]. The protein phosphatase POLTERGEIST (POL) [36] was isolated from a genetic screen for modifiers of the *clv* meristem phenotype [37]. Mutations in either *POL* or a closely related gene *PLL1* provide partial, additive suppression of the *clv* stem cell accumulation defect [38]. *POL*/*PLL1* over-expression inhibits differentiation and induces stem cell accumulation, especially in a *clv* background, suggesting that POL/PLL1 are intermediates downstream of the CLV1/CLV2 complex that indirectly promote *WUS* expression [39]. An additional component of the CLV complex is a Rho GTPase-related protein (Rop) that is related to the Ras GTPase superfamily in animals [32]. Ras GTPases are typically associated with cytosolic mitogen-activated protein kinase (MAPK) cascades [7], but whether such a kinase cascade lies downstream of the CLV1 receptor complex has not yet been determined.

The CLV pathway induces downstream signaling events that limit the size of the *WUS* expression domain. The *WUS* gene encodes a member of the WOX family of homeodomain transcription factors. *WUS* mRNA expression is confined to a group of cells in the deeper layers of the meristem, called the organizing center (OC), that specify the overlying cells as stem cells [40]. The *WUS* expression domain expands in *clv* shoot and floral meristems, indicating that *WUS* is negatively regulated by the CLV pathway (Fig. 3) [41]. In fact, over-expression of *CLV3* results in complete repression of *WUS* expression throughout the meristem and conditions a *wus*-like phenotype of

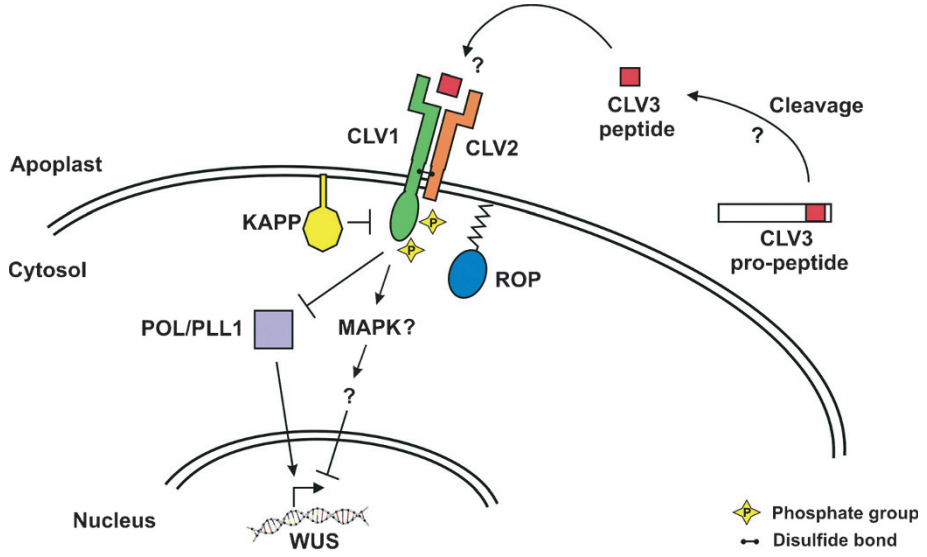

Figure 3. CLV3 signal transduction pathway. The CLV3 pro-peptide is secreted into the apoplast where it is likely processed to its mature form. CLV3 peptide acts as a ligand that is perceived by a CLV1 and CLV2 disulfide-linked heterodimeric receptor complex. In the presence of CLV3, the CLV1 kinase domain undergoes autophosphorylation and associates with the protein phosphatase KAPP, which can bind and dephosphorylate CLV1 and behaves as a negative regulator of the pathway. Another component of the active CLV complex is ROP, a Rho-like GTPase, the activation of which may initiate a MAPK signaling cascade to inhibit *WUS* at the transcriptional level. The largely redundant protein phosphatases POL and PLL1 act downstream in the pathway as putative positive regulators of *WUS* transcription that are attenuated by activity of the CLV complex.

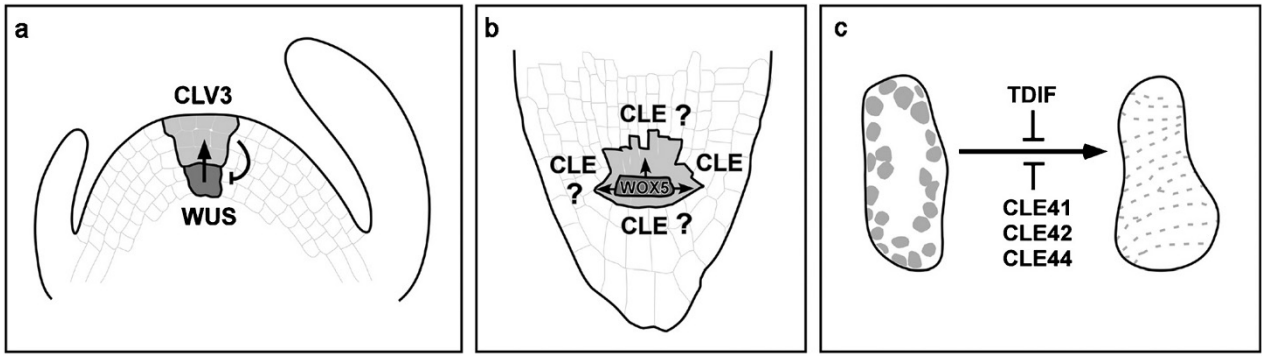

Figure 4. CLE functions in plants. (*a*) Schematic of the *Arabidopsis* shoot apical meristem. *CLV3* mRNA is restricted to stem cells in the outer layers of the central zone, whereas *WUS* expression is detected in a few central cells called the OC (organizing center) in the deeper layer of the meristem. MCLV3 is released from the stem cells and moves inward to activate a CLV1/CLV2 receptor complex to limit WUS activity by restricting its expression domain. Non-autonomous WUS activity confers stem cell identity and positively regulates *CLV3* expression. This CLV-WUS negative feedback loop confers stem cell homeostasis on the shoot apical meristem. (*b*) Schematic of the *Arabidopsis* root apical meristem. *WOX5* expression is restricted to the QC cells and is required for maintenance of the surrounding root meristem cells. Expression of several *CLE* genes has been reported in the root, one or more of which may regulate the *WOX5* expression domain via a CLV3-like signal transduction pathway. (*c*) Schematic of trans-differentiation in a *Zinnia in vitro* model system. Mesophyll-cell-containing green chloroplasts (left) isolated from a *Zinnia* leaf trans-differentiates into a TE (tracheary element, right) in response to auxin and cytokinin in an *in vitro* cell culture system. *Zinnia* TDIF as well as the *Arabidopsis* CLE41, CLE42 and CLE44 dodecapeptides can specifically inhibit stage two of TE differentiation.

premature SAM termination. Using a *CLV3-GFP* fusion construct, Lenhard and Laux showed that CLV3 protein spreads laterally and downward from the stem cells to their neighbors. This non-cell-autonomous effect of CLV3 can be abolished by coexpression of *CLV1,* indicating that CLV1 sequesters the ligand and protects the *WUS*-expressing OC from the effects of CLV3 signaling [42]. Conversely, *WUS* acts non-cell-autonomously to confer stem cell identity on the overlying cells and to promote *CLV3* expression by those cells [43]. Through this CLV-WUS negative feedback loop (Fig. 4), stem cell homeostasis is maintained in the shoot apical meristem.

Two live-imaging studies have revealed the dynamic activity of CLV3 in stem cell maintenance. One group used inducible *CLV3* over-expression to down-regu-

late *WUS* expression, which induced incorporation of the outermost cells of the CZ into organ primordia and resulted in a shift of the boundary between CZ and PZ identity [44]. Conversely, when *WUS* expression was released from regulation by *CLV3* in *CLV3* interference (*CLV3i*) lines, expansion of the CZ due to the respecification of PZ cells to adopt a CZ fate was detected [45]. These results suggest that the balance between WUS and CLV3 is required both to regulate stem cell proliferation and to position the CZ/PZ boundary in the SAM. In sum, the CLV3 polypeptide is a key developmental mediator required to communicate cell fate information between the stem cells and their neighbors in the shoot apical meristem.

FON4/FON2

Recent studies of the rice *FON4/FON2* gene suggest that the genetic mechanism underlying the regulation of shoot apical meristem maintenance by the CLV3 signaling pathway is conserved in other plant species [20, 46]. The *fon4/fon2* mutation was isolated from rice based on its shoot apical meristem enlargement and increased floral organ number phenotypes. FON4/FON2 appears to be the structural and functional counterpart of CLV3 in rice. The two proteins have similar genomic structures and high sequence homology in the CLE domain, and *FON4/FON2* mRNA transcripts are detected in a small group of cells at the apex of shoot and floral meristems, similar to *CLV3*. In addition, *FON4/FON2* activity rescues the *clv3* mutant phenotype and over-expression of *FON4/FON2* in wild-type *Arabidopsis* plants conditions a *CLV3* over-expression phenotype (Table 1).

Recently, the *FON1* gene encoding an LRR receptor-like kinase similar to CLV1 has been shown to function in rice floral meristem regulation [47]. Genetic interactions between *FON4/FON2* and *FON1* in rice as well as functional analysis of *FON4/FON2* activity in *fon1* plants clearly indicate that the rice FON4/FON2-FON1 pathway corresponds to the *Arabidopsis* CLV signaling system even though different effects of synthetic FON4p peptide and CLV3p peptide were observed in rice root apical meristems [20]. The difference in defects caused by the *fon1* and *fon4/2* mutations suggests that FON1 is not the only receptor for FON4/FON2. OsLRK1, a close relative of FON1, may be a candidate for an additional receptor, because plants containing an antisense version of OsLRK1 display an increased floral organ number phenotype characteristic of *fon4/fon2* and *fon1* plants [48].

Because *Arabidopsis* and rice are distantly related plant species, the mechanism of shoot and floral meristem size regulation by the CLV signaling pathway may be functionally conserved in a wide range of flowering plants. Consistent with this notion, mutations in *thick tassel dwarf1* (*td1*) or *fasciated ear2* (*fea2*) cause enormous enlargement of the maize female reproductive (ear) meristem, and *TD1* and *FEA2* encode the putative maize orthologs of CLV1 and CLV2, respectively [49, 50]. Yet although the maize mutants have highly enlarged reproductive shoot apical meristems, no reproductive SAM phenotype was observed in rice *fon1* mutants. Furthermore, the *td1* and *fea2* genes do not function in a single pathway [49], unlike the *Arabidopsis CLV1* and *CLV2* genes. Thus despite its fundamental importance to plant development, the molecular mechanism of shoot apical meristem maintenance is not completely identical between species.

CLE40

CLE40 together with *CLV3* are the only Arabidopsis *CLE* family members for which loss-of-function phenotypes have been reported [51]. *CLV3* and *CLE40* differ from the other family members in having an intron that lies just after the signal sequence, and in both genes the intron and the two exons are comparable in size. Despite the common organization at the DNA level, the parallel between *CLV3* and *CLE40* ends when other molecular and functional features are taken into consideration. RT-PCR and RNA *in situ* hybridization experiments detected weak and ubiquitous expression of *CLE40* mRNA in all tissues [23, 51]. A loss-of-function *cle40-En* allele was isolated that carries a transposable element insertion immediately upstream of the CLE domain [51]. No above-ground phenotypes were observed in *cle40-En* plants, indicating that *CLE40* does not play a significant role in the *CLV3*-mediated meristem regulatory pathway. However, *cle40-En* roots exhibited a slightly waving pattern, grew shorter and were strongly slanted to the left, indicative of defective gravity perception and/or response. This observation not only implies that *CLE40* function is important for proper root growth, but also suggests that the CLE40 signaling pathway could integrate with the signaling pathways of the phytohormones auxin and ethylene which are known to play a role in the response of roots to gravity.

TDIF/CLE41/CLE42/CLE44

Whereas CLV3 and its orthologs act to restrict stem cell accumulation, several other CLE family members have been shown to inhibit cell differentiation [52]. This function was first identified from *Zinnia elegans* mesophyll cell culture through the isolation of an extracellular factor that inhibited vascular tracheary element differentiation and promoted cell division. The isolated small proteinaceous factor was a CLE-

related dodecapeptide with two hydroxyproline (Hyp) residues – HEVHypSGHypNPISN – that was designated tracheary element differentiation inhibitory factor (TDIF). A full-length cDNA for *Zinnia* TDIF was isolated and found to encode a 132-amino-acid protein containing a 12-amino-acid sequence corresponding to TDIF at the carboxyl terminus. Thus TDIF, like CLV3, appears to be produced through post-translational processing and modification.

The CLE domains of the *Arabidopsis* CLE41 and CLE44 proteins are identical to that of TDIF, and the CLE domain of CLE42 differs in only one amino acid residue. When the synthetic 12-amino-acid polypeptides corresponding to various CLE proteins were exogenously applied to *Zinnia* cell culture, only those dodecapeptides corresponding to the predicted sequences from CLE41/CLE44 and CLE42 exhibit strong TDIF activity (Table 1). Unexpectedly, application of mature CLV3 polypeptide to this cell culture system has the opposite effect, promoting the differentiation of the tracheary element (TE) cells [52]. These observations reveal that two CLE pathways are active during *Zinnia* vascular development, one activated by TDIF that inhibits TE differentiation and one that can be activated by ectopic CLV3 that promotes TE differentiation.

Other CLE functions

The loss-of-function genetic approach is an extremely powerful tool in the identification of gene function. However, no functional information is available for the vast majority of the *CLE* genes because of their small size and the functional redundancy that can be anticipated based on the sequence similarity and overlapping expression domains of many *CLE* genes [23]. For example, no phenotypes were detected in *Arabidopsis* plants carrying a *CLE19* T-DNA null insertion allele [53], suggesting the possibility of a functionally redundant partner or partners among closely related CLE family members.

Although the fact that many *CLE* genes do not appear to be expressed in the shoot apical meristem [23] suggests that they do not function in SAM maintenance, many CLE proteins can activate the CLV3 signaling pathway when ectopically expressed in the SAM [19, 51, 54]. To date, ectopic expression of at least 12 Arabidopsis *CLE* genes produce phenotypes similar to those of *CLV3* over-expressing plants (Table 1), and a similar number completely or partially rescue the *clv3* phenotype. The ability of CLE1 to rescue the *clv3* phenotype was shown to be largely dependent on CLV1, whereas that of CLE22 was CLV1 independent [54]. This suggests that the CLE22 polypeptide is not recognized by the CLV1 receptor kinase. CLE4 and CLE40 also rescue the *clv3* phenotype in a putatively CLV1-independent fashion [19, 51]. However, these studies used strong *clv1* alleles that act in a dominant-negative manner, making the data difficult to interpret because the dominant-negative phenotype seems to mask the activity of functionally redundant receptors that also regulate shoot *Arabidopsis* stem cell accumulation [33].

Novel pathways that can be activated by *CLE* gene activity have been revealed through gain-of-function analysis. For example, in addition to shoot apical meristem arrest, plants ectopically expressing *CLV3* also exhibited a root apical meristem termination phenotype (Table 1). Similar phenotypes were observed in extensive studies of *CLE19* and *CLE40* transgenic plants [51, 53, 55]. Ectopic expression of *CLV3*, *CLE19* or *CLE40* under the control of the 35S or the RCH1 root-specific promoter in the root apical meristem caused progressive loss of root meristem cells and induction of cellular differentiation, as indicated by the formation of root hair cells closer than normal to the root tip. Application of chemically synthesized 14-amino-acid polypeptides, CLV3p, CLE19p, and CLE40p, corresponding to the conserved CLE motif, was sufficient to induce the root apical meristem consumption phenotype, whereas mutant CLE polypeptides engineered with single amino acid changes or deletions were not functional [56]. In recent bioassays using synthetic CLE dodecapeptides, all of the *CLE* genes except for *CLE1* through *CLE7*, *CLE41*, *CLE42* and *CLE44* showed strong inhibition of root meristem maintenance, suggesting that the CLE motif is the functional cue in this assay (Table 1) [52]. These data indicate that a CLE signaling pathway may control cell fate in the root (Fig. 4). This is also supported by the identification of the *WUS-RELATED HOMEOBOX* (*WOX*) gene *WOX5,* which is required for stem cell maintenance in the root quiescent center (QC) [57]. Given that *WOX5* is a homolog of *WUS* and that the QC has an analogous role in the root to the OC in the shoot, it has been reasonably proposed that conserved CLE-mediated signaling pathways may control stem cell activity in both the SAM and the root apical meristem (RAM). Interestingly, the CLE-induced root meristem termination phenotype appears to be dependent on CLV2 function [56], implying that CLV2 is involved in the perception of CLE polypeptides in roots. Since CLV2 has a relatively broad expression pattern, it will be interesting to determine if CLV2 is involved in a wide range of CLE-mediated signaling pathways.

Other *CLE* over-expression phenotypes have recently been characterized [19]. Plants that over-express *CLE19*, *CLE21*, or *CLE*25 display miniature rosettes

and inflorescences, often accompanied by developmental timing delays, apparent anthocyanin overproduction, and dwarfism. Plants over-expressing either *CLE42* or *CLE44* develop a shrub-like phenotype with small and rounded leaves and reduced apical dominance. Like the diverse *CLE* gene expression patterns, the variety of over-expression phenotypes indicate that CLE-activated signal transduction pathways are likely to operate in a number of *Arabidopsis* tissues. There seems to be a correlation between over-expression phenotype and CLE domain amino acid sequence, because many *CLE* genes in the same clade showed similar over-expression phenotypes [19]. However, because the CLE domain is highly conserved, it remains to be determined if the variable domain outside the CLE domain contributes to the specificity of any of the CLE proteins for receptor recognition and biological function.

Post-translational modifications and processing

A great deal of experimental evidence suggests that the CLE motif confers protein activity. The first clue toward this conclusion was furnished by the identification of two independent *CLV3* alleles, *clv3–1* and *clv3–5*. Both alleles carried a point mutation in the highly conserved glycine75 codon in the CLE domain (Fig. 1) that confers an intermediate *clv3* phenotype [16], revealing the importance of this domain for protein function. Moreover, application of synthetic 14-amino-acid polypeptides corresponding to the CLE motif of CLV3, CLE19 and CLE40 to *Arabidopsis* roots mimics the over-expression phenotype of these *CLE* genes [56]. CLV3 deletion analysis showed that only the signal peptide and the CLE motif are necessary for function [58]. Furthermore, Ni and Clark [54] designed CLE domain swap experiments in order to assess whether CLE proteins that are not normally able to replace CLV3 function could acquire this ability when their CLE domains were replaced by that of CLV3. They also constructed a chimeric protein in which the CLV3 signal peptide and non-conserved sequences were replaced with sequences from the unrelated ERECTA receptor kinase, leaving only the C-terminal domain from CLV3. In all cases the chimeric proteins that contained only the CLE domain from CLV3 were able to complement the *clv3* phenotype [54]. The outcome of these experiments strongly suggests that the CLE domain is the only functional region of the CLV3 protein.

Studies of two natural CLE peptides, TDIF in *Zinnia* and CLV3 in *Arabidopsis* [26, 52], clearly show that processing of full-length CLE protein into a 12-amino-acid polypeptide is crucial for function. This CLE dodecapeptide motif contains a number of conserved amino acids. Two or three proline residues are always present and a glycine residue at position 6 is highly conserved, while a valine residue at position 3 and two asparagine residues at positions 8 and 12 are less conserved. The glycine, valine and asparagine residues as well as a proline residue at position 9 seem to be crucial for *Zinnia* TDIF activity [52]. Although the isolated CLE peptides were hydroxylated at two proline residues, this post-translational modification seems not to be necessary for activity [26, 52].

Although the mechanism through which the CLE proteins are processed has not yet been determined, a few hints have been gleaned from biochemical and genetic studies. First, a non-membrane-associated protease activity that can process both CLV3 and CLE1 has been detected in cauliflower extracts [54]. Second, a suppressor of *CLE19* over-expression, *SOL1,* has been identified that encodes a putative Zn^{2+}-carboxypeptidase [55]. Animal proteins homologous to SOL1 have been shown to cleave terminal R and K residues and play roles in prohormone and neuropeptide processing. These findings seem to suggest that some plant signaling molecules, like their animal analogs, are synthesized as inactive protein precursors from which the active molecules are liberated by proteolysis.

CLE genes in other organisms

CLE genes have been identified in the genomic and EST databases [18] of many different plant species, including *Brassica*, maize, tomato, soybean, *Medicago*, rice, cotton and wheat. There has not been an effort to identify all the CLE family members within plant species other than *Arabidopsis*, although recently 13 CLE family members were uncovered in the rice genome [20]. Three *CLE* genes have been identified thus far in maize – *Esr1*, *Esr2* and *Esr3* – which are 80 % to 90 % homologous to one another at the nucleotide level [59]. They also share very similar expression patterns, being confined to the portion of the endosperm that surrounds the embryo between 4 and 28 days after pollination [60]. Although the roles of these proteins are not known, it is postulated that they are involved in signaling between the endosperm and embryo during early maize development. Six *CLE* genes have been recently identified in the lycophyte *Selaginella moellendorffii* and one in the moss *Physcomitrella patens* [61], suggesting that the family originated soon after the evolution of land plants.

Although the *CLE* gene family is considered plant specific, a CLE-like protein called HG-SYV46 has

been discovered in the soybean cyst nematode *Heterodera glycines* [62, 63]. HG-SYV46 is believed to be an example of convergent evolution, and more specifically of adaptive molecular mimicry [62]. It is thought to be secreted by the parasitic nematode after its penetration in the soybean roots, in order to imitate the function of an endogenous CLE peptide and induce root cell proliferation and the consequent formation of syncytial feeding sites.

Perspectives and Frontiers

Many advances in the past few years have shed light on aspects of *CLE* gene structure, expression, activity and function. Even based on the limited functional data available, it seems clear that CLE family members play roles in a variety of activities during plant growth and development. Interestingly, some CLE proteins appear to be functionally equivalent and capable of activating the same pathways when ectopically expressed, suggesting that the specificity of CLE functions is mainly achieved by differential expression patterns (sub-functionalization at the level of regulatory sequences) rather than at the biochemical level (neo-functionalization). Given our knowledge of *CLE* gene expression patterns and the functional roles of a few of them, it seems reasonable to expect that a large number of different plant biological processes are under the control of this gene family.

The CLE family and other families of small putative signaling molecules are unique to plants, whereas, conversely, well-known animal signaling molecules such as Wnt, DPP, TGF-β and EGF have no plant homologs. Nonetheless, the general molecular mechanisms of signal transduction within the cell via intermediates such as receptor kinases, protein phosphatases and Ras superfamily members are shared between the two kingdoms. Thus, plants and animals have evolved similar strategies for transducing intercellular peptide signals, but the signaling molecules themselves appear to have been derived independently.

The identification of downstream CLE signaling components remains a fundamental unresolved issue because very few players have been identified to date. At the moment, only CLV3 can be placed in a signaling pathway where a number of components are known. One major issue to be addressed is the identification of receptors. It is widely speculated that the RLK family represents the major receptor system for intercellular signaling in plants, due to the fact that it contains more than 400 members in *Arabidopsis* [64] and largely overshadows the other plant receptor kinase family, the histidine kinases [65]. On this premise, and on the basis of the CLV paradigm, RLKs are the primary candidate receptors for the CLE proteins. Some putative ligand-receptor pairs may be guessed at by comparing their expression patterns, because it is known that the CLV3 polypeptide moves only a few cell layers [42], so candidate ligands and receptors are expected to be expressed in close proximity to each other. However, it is quite possible that many CLE receptors will have much broader expression domains than their ligands, and could possibly interact with different CLE proteins in different tissues. Future studies will undoubtedly focus on the genetic and biochemical identification of receptor-ligand pairs through suppressor screens, pull-down assays, coimmunoprecipitation and bimolecular fluorescence complementation.

Many intercellular signaling components may be shared by multiple CLE pathways, although their downstream targets are likely to be unique. It is already known that CLV2 has a functional role in both the shoot and roots, and that the protein phosphatase KAPP interacts with both CLV1 and a related RLK called HAESA [66]. Furthermore, the ubiquitous expression of *POL* [36] raises the possibility that this protein phosphatase may be recruited into different CLE signal pathways. Other plant LRR-RLKs use MAPK pathways for intercellular signal transduction, and this may be the case for the CLE-mediated signaling pathways as well. Another CLV3 pathway component, WUS, has homologs that could act as downstream targets of other CLE signaling pathways, and *WOX* genes have been shown to function in flower development [67], cell division maintenance [68], root stem cell signaling [57] and embryo development [69]. Determining the extent of functional overlap between the *CLE* gene family and the *WOX* gene family represents an important opportunity for the future.

Another unresolved fundamental issue concerning *CLE* gene function is the degree of redundancy among family members. Except for *CLV3* and *CLE40* [16, 51], the failure to observe a distinguishable phenotype in other *CLE* loss-of-function mutants [53] has been interpreted as a symptom of full genetic redundancy. However, it must be noted that few *CLE* null mutants have been identified, probably due to their small gene size, and future mutant identification could revert this notion. Another possible explanation for the lack of loss-of-function phenotypes is the failure to detect them in a laboratory setting because they are conditional to environmental stimuli. Furthermore, it should be taken into consideration that mutant screens often focus on obvious strong phenotypes and do not always cover the whole plant life cycle. In cases where redundancy proves relevant to

the study of *CLE* gene function, a number of strategies can be pursued, such as the generation of higher-order mutant combinations and the construction of artificial miRNA and RNAi vectors targeting multiple *CLE* genes at the same time.

Finally, an as yet unexplored avenue of investigation is the extent to which the CLE polypeptide signaling pathways intersect and integrate with the phytohormone signaling pathways. Phytohormones such as auxin and cytokinin are known to act systemically, but also to produce a range of tissue- and stage-specific biological effects. The available evidence suggests that CLV3 and other CLE peptides may act over a much shorter range and could potentially refine the more global phytohormone signals into dynamic, coordinated decisions among neighboring groups of cells. Fortunately, work on the *CLE* gene family is progressing rapidly, and further studies will undoubtedly yield new key insights into the mechanisms of cell-to-cell communication in plants.

Acknowledgements. Work in our laboratory is funded by the United States Department of Agriculture and the National Science Foundation.

1 Bliss, M. (1982) The Discovery of Insulin, University of Chicago Press, Chicago.
2 Brivanlou, A. H. and Darnell Jr., J. E. (2002) Signal transduction and the control of gene expression. Science 295, 813–818.
3 van der Geer, P., Hunter, T. and Lindberg, R. A. (1994) Receptor protein-tyrosine kinases and their signal transduction pathways. Annu. Rev. Cell Biol. 10, 251–337.
4 Feng, X.-H. and Derynck, R. (2005) Specificity and versatility in TGF-β signaling through SMADS. Annu. Rev. Cell Dev. Biol. 21, 659–693.
5 Harrisa, R. C., Chunga, E. and Coffey, R. J. (2003) EGF receptor ligands. Exp. Cell Res. 284, 2–13.
6 Todorovica, V., Jurukovskia, V., Chena, Y., Fontana, L., Dabovica, B. and Rifkin, D. B. (2005) Latent TGF-β binding proteins. Int. J. Biochem. Cell Biol. 37, 38–41.
7 Downward, J. (2001) The ins and outs of signaling. Nature 411, 759–762.
8 Gaspar, T. H., Kevers, C., Faivre-Rampant, O., Crevecoeur, M., Penel, C. L., Greppin, H. and Dommes, J. (2003) Changing concepts in plant hormone action. In Vitro Cell. Dev. Biol. Plant 39, 85–106.
9 Pearce, G., Strydom, D., Johnson, S. and Ryan, C. A. (1991) A polypeptide from tomato leaves activates the expression of proteinase inhibitor genes. Science 253, 895–898.
10 McGurl, B., Pearce, G., Orozco-Cardenas, M. and Ryan, C. A. (1992) Structure, expression, and antisense inhibition of the systemin precursor gene. Science 255, 1570–1573.
11 Matsubayashi, Y. and Sakagami, Y. (2006) Peptide hormones in plants. Annu. Rev. Plant Biol. 57, 649–674.
12 Matsubayashi, Y. and Sakagami, Y. (1996) Phytosulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of *Asparagus officinalis* L. Proc. Natl. Acad. Sci. USA 93, 7623–7627.
13 Casson, S. A., Chilley, P. M., Topping, J. F., Evans, I. M., Souter, M. A. and Lindsey, K. (2002) The POLARIS gene of *Arabidopsis* encodes a predicted peptide required for correct root growth and leaf vascular patterning. Plant Cell 14, 1705–1721.
14 Butenko, M. A., Patterson, S. E., Grini, P. E., Stenvik, G. E., Amundsen, S. S., Mandal, A. and Aalen, R. B. (2003) Inflorescence deficient in abscission controls floral organ abscission in Arabidopsis and identifies a novel family of putative ligands in plants. Plant Cell 15, 296–307.
15 Narita, N. N., Moore, S., Horiguchi, G., Kubo, M., Demura, T., Fukuda, H., Goodrich, J. and Tsukaya, H. (2004) Overexpression of a novel small peptide ROTUNDIFOLIA4 decreases cell proliferation and alters leaf shape in Arabidopsis thaliana. Plant J. 38, 699–713.
16 Fletcher, J. C., Brand, U., Running, M. P., Simon, R. and Meyerowitz, E. M. (1999) Signaling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems. Science 283, 1911–1914.
17 Opsahl-Ferstad, H. G., Le Deunff, E., Dumas, C. and Rogowsky, P. M. (1997) ZmEsr, a novel endosperm-specific gene expressed in a restricted region around the maize embryo. Plant J. 12, 235–246.
18 Cock, J. M. and McCormick, S. (2001) A large family of genes that share homology with CLAVATA3. Plant Physiol. 126, 939–942.
19 Strabala, T. J., O'Donnell, P. J., Smit, A.-M., Ampomah-Dwamena, C., Martin, E. J., Netzler, N., Nieuwenhuizen, N. J., Quinn, B. D., Foote, H. C.C. and Hudson, K. R. (2006) Gain-of-function phenotypes for many *CLAVATA3/ESR* genes, including four new family members, correlate with tandem variations in the conserved *CLAVATA3/ESR* domain. Plant Physiol. 140, 1331–1344.
20 Chu, H., Qian, Q., Liang, W., Yin, C., Tan, H., Yao, X., Yuan, Z., Yang, J., Huang, H., Luo, D., Ma, H. and Zhang, D. (2006) The *FLORAL ORGAN NUMBER4* gene encoding a putative ortholog of Arabidopsis CLAVATA3 regulates apical meristem size in rice. Plant Physiol. 142, 1039–1052.
21 Clark, S. E., Running, M. P. and Meyerowitz, E. M. (1995) CLAVATA3 is a specific regulator of shoot and floral meristem development affecting the same processes as CLAVATA1. Development 121, 2057–2067.
22 Bowers, J. E., Chapman, B. A., Rong, J. and Paterson, A. H. (2003) Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. Nature 422, 433–438.
23 Sharma, V. K., Ramirez, J. and Fletcher, J. C. (2003) The Arabidopsis CLV3-like (CLE) genes are expressed in diverse tissues and encode secreted proteins. Plant Mol. Biol. 51, 415–425.
24 Clark, S. E. (2001) Cell signalling at the shoot meristem. Nat. Rev. Mol. Cell. Biol. 2, 276–284.
25 Rojo, E., Sharma, V. K., Kovaleva, V., Raikhel, N. V. and Fletcher, J. C. (2002) CLV3 is localized to the extracellular space, where it activates the *Arabidopsis* CLAVATA stem cell signaling pathway. Plant Cell 14, 969–977.
26 Kondo, T., Sawa, S., Kinoshita, A., Mizuno, S., Kakimoto, T., Fukuda, H. and Sakagami, Y. (2006) A plant peptide encoded by CLV3 identified by in situ MALDI-TOF MS analysis. Science 313, 845–848.
27 Rehemtulla, A. and Kaufman, R. J. (1992) Protein processing within the secretory pathway. Curr. Opin. Biotechnol. 3, 560–565.
28 Clark, S. E., Running, M. P. and Meyerowitz, E. M. (1993) CLAVATA1, a regulator of meristem and flower development in *Arabidopsis*. Development 119, 397–418.
29 Kayes, J. M. and Clark, S. E. (1998) CLAVATA2, a regulator of meristem and organ development in *Arabidopsis*. Development 125, 3843–3851.
30 Clark, S. E., Williams, R. W. and Meyerowitz, E. M. (1997) The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis. Cell 89, 575–585.
31 Jeong, S., Trotochaud, A. E. and Clark, S. E. (1999) The Arabidopsis CLAVATA2 gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase. Plant Cell 11, 1925–1933.

32 Trotochaud, A. E., Hao, T., Wu, G., Yang, Z. and Clark, S. E. (1999) The CLAVATA1 receptor-like kinase requires CLAVATA3 for its assembly into a signaling complex that includes KAPP and a Rho-related protein. Plant Cell 11, 393–405.
33 Dievart, A., Dalal, M., Tax, F. E., Lacey, A. D., Huttly, A., Li, J. and Clark, S. E. (2003) CLAVATA1 dominant-negative alleles reveal functional overlap between multiple receptor kinases that regulate meristem and organ development. Plant Cell 15, 1198–1211.
34 Stone, J. M., Trotochaud, A. E., Walker, J. C. and Clark, S. E. (1998) Control of meristem development by CLAVATA1 receptor kinase and kinase-associated phosphatase interactions. Plant Physiol. 117, 1217–1225.
35 Williams, R. W., Wilson, J. M. and Meyerowitz, E. M. (1997) A possible role for kinase-associated protein phosphatase in the *Arabidopsis* CLAVATA1 signaling pathway. Proc. Natl. Acad. Sci. USA 94, 10467–10472.
36 Yu, L. P., Miller, A. K. and Clark, S. E. (2003) POLTERGEIST encodes a protein phosphatase 2C that regulates the CLAVATA pathways controlling stem cell identity at Arabidopsis shoot and floral meristems. Curr. Biol. 13, 179–188.
37 Pogany, J. A., Simon, E. J., Katzman, R. B., de Guzman, B. M., Yu, L. P., Trotochaud, A. E. and Clark, S. E. (1998) Identifying novel regulators of shoot meristem development. J. Plant Res. 111, 307–313.
38 Yu, L. P., Simon, E. J., Trotochaud, A. E. and Clark, S. E. (2000) POLTERGEIST functions to regulate meristem development downstream of the CLAVATA loci. Development 127, 1661–1670.
39 Song, S.-K., Lee, M. M. and Clark, S. E. (2006) POL and PLL1 phosphatases are CLAVATA1 signaling intermediates required for *Arabidopsis* shoot and floral stem cells. Development 133, 4691–4698.
40 Mayer, K. F., Schoof, H., Haecker, A., Lenhard, M., Jurgens, G. and Laux, T. (1998) Role of WUSCHEL in regulating stem cell fate in the *Arabidopsis* shoot meristem. Cell 95, 805–815.
41 Brand, U., Fletcher, J. C., Hobe, M., Meyerowitz, E. M. and Simon, R. (2000) Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by CLV3 activity. Science 289, 617–619.
42 Lenhard, M. and Laux, T. (2003) Stem cell homeostasis in the *Arabidopsis* shoot meristem is regulated by intercellular movement of CLAVATA3 and its sequestration by CLAVATA1. Development 130, 3163–3173.
43 Schoof, H., Lenhard, M., Haecker, A., Mayer, K. F., Jurgens, G. and Laux, T. (2000) The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. Cell 100, 635–644.
44 Muller, R., Borghi, L., Kwiatkowska, D., Laufs, P. and Simon, R. (2006) Dynamic and compensatory responses of *Arabidopsis* shoot and floral meristems to CLV3 signaling. Plant Cell 18, 1188–1198.
45 Reddy, G. V. and Meyerowitz, E. M. (2005) Stem-cell homeostasis and growth dynamics can be uncoupled in the *Arabidopsis* shoot apex. Science 310, 663–667.
46 Suzaki, T., Toriba, T., Fujimoto, M., Tsutsumi, Y., Kitano, H. and Hirano, H. (2006) Conservation and diversification of meristem mechanism in *Oryza sativa:* function of the *FLORAL ORGAN NUMBER2* gene. Plant Cell Physiol. 47, 1591–1602.
47 Suzaki, T., Sato, M., Ashikari, M., Miyoshi, M., Nagato, Y. and Hirano, H. Y. (2004) The gene FLORAL ORGAN NUMBER1 regulates floral meristem size in rice and encodes a leucine-rich repeat receptor kinase orthologous to *Arabidopsis* CLAVATA1. Development 131, 5649–5657.
48 Kim, C., Jeong, D.-H. and An, G. (2000) Molecular cloning and characterization of *OsLRK1* encoding a putative receptor-like protein kinase from *Oryza sativa.* Plant Sci. 12, 17–26.
49 Bommert, P., Lunde, C., Nardmann, J., Vollbrecht, E., Running, M. P., Jackson, D., Hake, S. and Werr, W. (2005) thick tassel dwarf1 encodes a putative maize ortholog of the *Arabidopsis* CLAVATA1 leucine-rich repeat receptor-like kinase. Development 132, 1235–1245.
50 Taguchi-Shiobara, F., Yuan, Z., Hake, S. and Jackson, D. (2001) The fasciated ear2 gene encodes a leucine-rich repeat receptor-like protein that regulates shoot meristem proliferation in maize. Genes Dev. 15, 2755–2766.
51 Hobe, M., Muller, R., Grunewald, M., Brand, U. and Simon, R. (2003) Loss of CLE40, a protein functionally equivalent to the stem cell restricting signal CLV3, enhances root waving in *Arabidopsis.* Dev. Genes Evol. 213, 371–381.
52 Ito, Y., Nakanomyo, I., Motose, H., Iwanoto, K., Sawa, S., Dohmae, N. and Fukuda, H. (2006) Dodeca-CLE peptides as suppressors of plant stem cell differentiation. Science 313, 842–845.
53 Fiers, M., Hause, G., Boutilier, K., Casamitjana-Martinez, E., Weijers, D., Offringa, R., van der Geest, L., van Lookeren Champagne, M. and Liu, C.-M. (2004) Mis-expression of the *CLV3/ESR*-like gene *CLE19* in *Arabidopsis* leads to a consumption of root meristem. Gene 327, 37–49.
54 Ni, J. and Clark, S. E. (2006) Evidence for functional conservation, sufficiency, and proteolytic processing of the CLAVATA3 CLE domain. Plant Physiol. 140, 726–733.
55 Casamitjana-Martinez, E., Hofhuis, H. F., Xu, J., Liu, C.-M., Heidstra, R. and Scheres, B. (2003) Root-specific *CLE19* overexpression and the *sol1/2* suppressors implicate a CLV-like pathway in the control of *Arabidopsis* root meristem maintenance. Curr. Biol. 13, 1435–1441.
56 Fiers, M., Golemiec, E., Xu, J., van der Geest, L., Heidstra, R., Stiekema, W. and Liu, C.-M. (2005) The 14-amino acid CLV3, CLE19, and CLE40 peptides trigger consumption of the root meristem in *Arabidopsis* through a *CLAVATA2*-dependent pathway. Plant Cell 17, 2542–2553.
57 Sakar, A. K., Luijten, M., Miyashima, S., Lenhard, M., Hashimoto, T., Nakajima, K., Scheres, B., Heidstra, R. and Laux, T. (2007) Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. Nature 446, 811–814.
58 Fiers, M., Golemiec, E., van der Schors, R., van der Geest, L., Li, K. W., Stiekema, W. J. and Liu, C.-M. (2006) The CLAVATA3/ESR motif of CLAVATA3 is functionally independent from the nonconserved flanking sequences. Plant Physiol. 141, 1284–1292.
59 Bonello, J. F., Sevilla-Lecoq, S., Berne, A., Risueno, M. C., Dumas, C. and Rogowsky, P. M. (2002) Esr proteins are secreted by the cells of the embryo surrounding region. J. Exp. Bot. 53, 1559–1568.
60 Bonello, J. F., Opsahl-Ferstad, H. G., Perez, P., Dumas, C. and Rogowsky, P. M. (2000) Esr genes show different levels of expression in the same region of maize endosperm. Gene 246, 219–227.
61 Floyd, S. K., and Bowman, J. L. (2007) The ancestral developmental tool kit of land plants. Int. J. Plant. Sci. 168, 1–35.
62 Olsen, A. N. and Skriver, K. (2003) Ligand mimicry? Plant-parasitic nematode polypeptide with similarity to CLAVATA3. Trends Plant Sci. 8, 55–57.
63 Wang, X., Mitchum, M. G., Gao, B., Li, C. M., Diab, H., Baum, T. J., Hussey, R. H. and Davis, E. L. (2005) A parasitism gene from a plant-parasitic nematode with function similar to *CLAVATA3/ESR (CLE)* of *Arabidopsis thaliana.* Mol. Plant Pathol. 6, 187–191.
64 Shiu, S. H. and Bleecker, A. B. (2001) Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. Proc. Natl. Acad. Sci. USA 98, 10763–10768.
65 Bleecker, A. B. and Kende, H. (2000) Ethylene: a gaseous signal molecule in plants. Annu. Rev. Cell Dev. Biol. 16, 1–18.
66 Stone, J. M., Collinge, M. A., Smith, R. D., Horn, M. A. and Walker, J. C. (1994) Interaction of a protein phosphatase with an *Arabidopsis* serine-threonine receptor kinase. Science 266, 793–795.

67 Matsumoto, N. and Okada, K. (2001) A homeobox gene, PRESSED FLOWER, regulates lateral axis-dependent development of *Arabidopsis* flowers. Genes Dev. 15, 3355–3364.
68 Wu, X., Dabi, T. and Weigel, D. (2005) Requirement of homeobox gene STIMPY/WOX9 for *Arabidopsis* meristem growth and maintenance. Curr. Biol. 15, 436–440.
69 Haecker, A., Grob-Hardt, R., Geiges, B., Sarkar, A., Breuninger, H., Herrmann, M. and Laux, T. (2004) Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana*. Development 131, 657–668.
70 Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl. Acids Res. 32, 1792–1797.
71 Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.