Front. Biol. 2017, 12(6): 406–420
DOI 10.1007/s11515-017-1468-9

REVIEW

CLE peptide-mediated signaling in shoot and vascular meristem development

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BACKGROUND: Multicellular organisms rely on the transmission of information between cells to coordinate various biological processes during growth and development. Plants, like animals, utilize small peptide ligands as signaling molecules to transmit information between cells. These polypeptides typically act as extracellular messengers that are perceived by membrane-bound receptors, which then transduce the signal into the recipient cell to modify downstream gene transcription. The CLAVATA3/EMBRYO SURROUNDING REGION-RELATED (CLE) proteins represent one of the largest and best understood families of small polypeptides in plants. Members of the CLE family play critical roles in mediating cell fate decisions during plant development, particularly within the unique meristem structures that contain stem cell reservoirs acting as sources of cells for continuous organ formation.

OBJECTIVE: Here we review the roles of CLE family members in regulating the activity of the shoot apical meristems that generate the aerial parts of the plants, and of the vascular meristems that produce the sugar- and water-conducting tissues.

METHODS: A systematic literature search was performed using the Google Scholar and PubMed search engines. The keywords “CLE”, “CLV3”, “TDIF”, “meristem”, and “plant stem cells” were used as search terms. The 95 retrieved articles, dating from 1992, were organized by topic and their key findings incorporated into the text.

RESULTS: We summarize our current understanding of how the CLE peptide CLV3 orchestrates the activity of shoot apical meristems, describing its expression, processing and movement, as well as its intracellular signal transduction pathways, key target genes and downstream gene regulatory networks. We also discuss the roles of CLE peptide signaling in the vascular meristems to promote procambial cell proliferation and suppress xylem differentiation.

CONCLUSIONS: Signaling pathways mediated by CLE peptides are critical for stem cell maintenance and differentiation in shoot apical and vascular meristems in plants, exposing *CLE* genes as potential targets for increasing yield and biomass production. While large numbers of *CLE* genes are being discovered in plants, only a few have been functionally characterized. We anticipate that future research will continue to elucidate the roles of the CLE family in plant development, and their potential impacts on agriculture and commerce.

Keywords CLE, CLV3, TDIF, WUS, stem cells, procambium

Introduction

The growth and development of multicellular organisms is heavily dependent on communication between groups of cells. Intercellular signaling pathways convey cell fate information, regulate cell division and differentiation processes, propagate and amplify specific signaling states, and coordinate tissue functions. Both plants and animals utilize systemic hormones as well as polypeptide signaling molecules to mediate cell-to-cell communication. In animals, polypeptides such as epidermal growth factor (EGF) and transforming growth factor-beta (TGF-β) act as extracellular ligands that are generated in certain cell types and perceived at the surface of neighboring cells, typically by transmembrane receptor kinases. Binding of the ligand to its receptor or receptors initiates a cascade of intracellular phosphorylation events that affects the activity of one or more nuclear transcription factors, resulting in the alteration of gene expression programs (Bergeron et al., 2016).

Although plants lack canonical EGF, TGF-β, Wingless and other peptide superfamilies found in animals, they also make

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Received July 19, 2017; accepted October 12, 2017

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extensive use of polypeptide signaling systems to mediate various biological processes (Matsubayashi, 2014; Tavormina et al., 2015). The genome of *Arabidopsis thaliana*, a member of the mustard family related to food plants such as broccoli and cauliflower, encodes well over a thousand small proteins (< 100 amino acids) that may function as peptide signaling molecules as well as more than 600 putative plasma membrane-bound receptor proteins (Shiu and Bleecker, 2001; Lease and Walker, 2006; Tavormina et al., 2015). The CLAVATA3/EMBRYO SURROUNDING REGION-RELATED (CLE) family represents one of the largest families of plant polypeptides identified to date, consisting of 32 members in *Arabidopsis* (Cock and McCormick, 2001) and as many as 84 members in other species (Hastwell et al., 2015). Members of the *CLE* gene family are present throughout the land plant lineage and in some plant parasitic nematodes (Cock and McCormick, 2001; Wang et al., 2005).

The *CLE* genes encode polypeptides of less than 15 kDa in molecular mass that share several structural features. Each peptide consists of either an N-terminal signal peptide or a membrane anchor sequence, a 40- to 90-amino acid variable domain, and a highly conserved 14-amino acid motif near the carboxyl terminus called the CLE domain (Cock and McCormick, 2001) (Fig. 1A). The signal peptide is sufficient to direct the CLE proteins through the secretory pathway (Rojo et al., 2002; Sharma et al., 2003) and is required for their *in vivo* function (Meng et al., 2010). Full length CLE pre-propeptides are processed (Casamitjana-Martínez et al., 2003; Ni and Clark, 2006) to produce biologically active 12-13 amino acid polypeptides consisting of the CLE domain (Kondo et al., 2006; Ohyama et al., 2009). The roles of the *CLE* genes are best understood in plant development and particularly in the meristems, which are small stem cell reservoirs that provide cells for continuous organ formation. Here we review recent insights into *CLE* gene regulation and function in the shoot apical meristem as well as in the meristematic tissue of the shoot vascular cambium.

SAM maintenance requires intercellular communication

The shoot apical meristem (SAM) of angiosperm plants is a small, highly organized structure at the growing shoot tip that provides all of the cells to generate the above ground architecture of the plant (Fig. 2). The SAM is established during embryogenesis and is maintained throughout the life of the plant. Its two major functions are to continuously initiate organs such as leaves and flowers and to sustain a stem cell reservoir for future organ formation. The organs arise as primordia on the flanks of the meristem, while at the apex the self-renewing stem cell reservoir replenishes the cells that have become incorporated into the organ primordia. To function as a site of ongoing organ formation, the SAM maintains a continuous balance between loss of stem cells through differentiation and their replacement through cell division.

The SAM is stratified into distinct cell layers called the tunica and corpus (Satina et al., 1940). In *Arabidopsis* and many other dicotyledonous plants, the tunica is comprised of

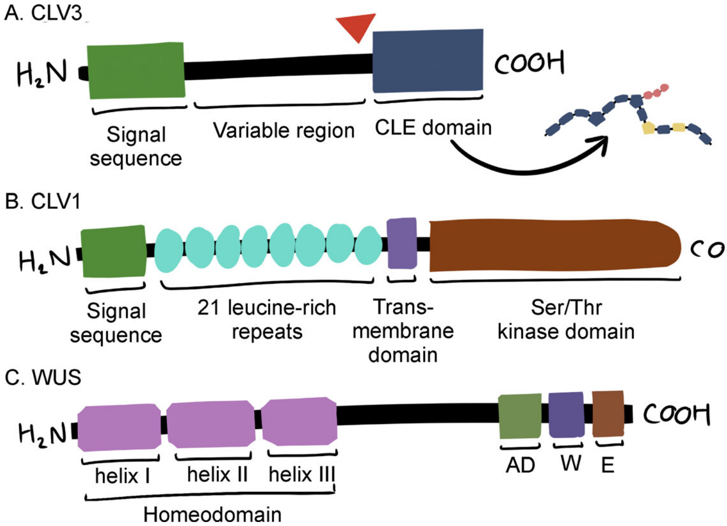

Figure 1 Functional domains of key SAM regulatory proteins. (A) The CLV3 pre-propeptide contains a signal sequence that directs it into the extracellular space, a variable region, and a CLE domain. The pre-propeptide is cleaved at the N terminus of the CLE domain (red arrowhead), and the released 12-13 amino-acid peptide is modified to generate a functional ligand. (B) The CLV1 protein is a receptor-like kinase (RLK) that consists of a signal sequence that directs it to the plasma membrane, 21 extracellular leucine-rich repeat domains, a transmembrane domain, and a cytosolic serine/threonine kinase domain. (C) The WUS protein is a transcription factor that contains an N-terminal DNA binding homeodomain consisting of three helices, as well as three conserved sequence motifs at the carboxyl-terminus: an acidic domain (AD), a WUS box (W), and an EAR domain (E). Molecules are not drawn to scale.

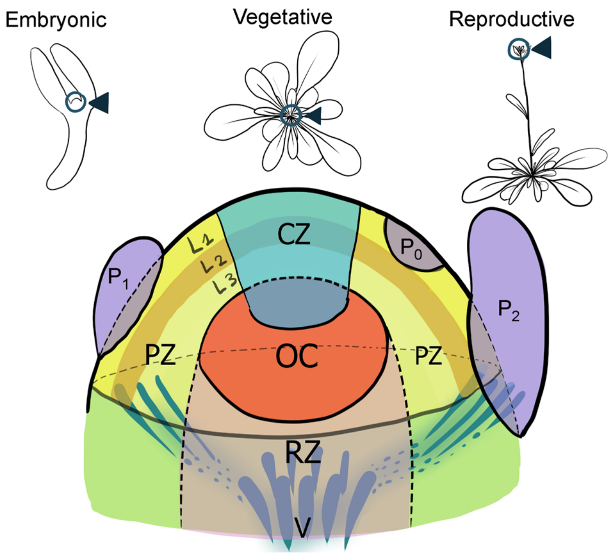

Figure 2 Organization of the *Arabidopsis thaliana* shoot apical meristem (SAM). The SAM is located at the growing shoot tip of the plant (blue circle and black arrowhead mark the location of the SAM in plants at three successive developmental stages). L1, L2, and L3 denote layers of distinct cell lineages. The central zone (CZ) is located at the apex and consists of stem cells that divide slowly into the surrounding peripheral zone (PZ) and the underlying rib zone (RZ). Cells in the PZ divide more rapidly and are recruited to form organ primordia (P_0, P_1, and P_2) or stem tissue. The organizing center (OC) at the top of the RZ functions as a niche that maintains stem cell identity in the CZ. Also shown is the vasculature (V) forming underneath the SAM and toward the developing organs.

an overlying L1 epidermal layer and a sub-epidermal L2 layer (Gifford, 1954). These layers are a single cell thick and remain clonally distinct from one another due to their specific cell division patterns (Poethig, 1987). The corpus, or L3, lies beneath the tunica and consists of cells that divide in all planes. Because cells in each layer participate in both SAM maintenance and organ formation (Poethig and Sussex, 1985a, b), these activities must be coordinated between all of the cell layers.

The SAM is also organized into three distinct functional domains. The central zone (CZ) at the very apex of the SAM consists of a reservoir of pluripotent stem cells with low mitotic activity (Steeves and Sussex, 1989). Divisions of stem cells in the CZ continuously displace their descendants outward into the surrounding peripheral zone (PZ) or downward into the interior rib zone (RZ). The PZ is a transitional region wherein more rapidly dividing stem cell descendants acquire more specified fates and become incorporated either into organ primordia or into regions of stem between the organs. The upper region of the RZ contains the organizing center (OC), which acts as a niche that sustains the overlying stem cell population. Cells in the RZ constitute the meristem pith and contribute to the bulk of the stem and vascular tissue (Steeves and Sussex, 1989). Classical experiments have demonstrated that the functional domains within the SAM exchange cell fate information (Sussex, 1954) and that the fate of each SAM cell is determined by positional information from the surrounding cells rather than from a lineage-specific heritage (Poethig et al., 1986; Furner and Pumfrey, 1992; Irish and Sussex, 1992). Thus SAM cells are in continuous communication with their neighbors in order to assess their relative positions in the meristem and behave accordingly.

A CLV3-mediated shoot apical meristem maintenance pathway

A molecular network called the CLAVATA (CLV)-WUSCHEL (WUS) pathway communicates cell fate decisions in the SAM and is essential for stem cell maintenance in higher plants (Somssich et al., 2016) (Fig. 3). The *Arabidopsis CLV3* gene is a founding member of the *CLE* gene family that is expressed exclusively within the stem cell reservoir of shoot apical and floral meristems (Fletcher et al., 1999). *CLV3* expression in the SAM initiates during the early heart stage of embryogenesis and continues throughout the life cycle. Loss-of-function mutations in *CLV3* cause an accumulation of supernumerary stem cells that leads to progressive SAM enlargement, resulting in the formation of strap-like fasciated stems that produce many more flowers than normal (Clark et al., 1995). Flowers arise from transient stem cell reservoirs in floral meristems (FM), which are also enlarged in *clv3* plants and produce extra floral organs. Thus *CLV3* restricts above ground stem cell accumulation throughout the life of the plant. Live imaging experiments revealed that *CLV3* performs this function both by restricting stem cell fate to the CZ and also by non-cell autonomously limiting cell division rates in the PZ (Reddy and Meyerowitz, 2005).

CLV3 encodes a secreted signaling molecule that is localized to the extracellular space (Fletcher et al., 1999; Rojo et al., 2002). As with many animal polypeptides, the CLV3 ligand is generated from a larger pre-propeptide that undergoes proteolytic cleavage of the signal peptide and the pro-domain before displaying biological activity. Cleavage of CLV3 occurs between the Leu^{69} and Arg^{70} residues (Ni and Clark, 2006; Ni et al., 2011; Xu et al., 2013), and requires a recognition domain of five amino acids flanking the N terminus of the CLE domain (Xu et al., 2013). It has been suggested that CLV3 cleavage is catalyzed by a serine protease (Ni et al., 2011), but this remains to be confirmed experimentally.

The active form of CLV3 was first identified as a 12 amino acid glycopeptide consisting of Arg^{70} to His^{81} of the CLE motif, in which the first two proline residues are modified to hydroxyproline (Kondo et al., 2006). When applied to *Arabidopsis* seedlings this synthetic MCLV3 peptide generates a SAM termination phenotype characteristic of *CLV3* gain-of-function plants, demonstrating its biological activity. Two studies have examined the contributions of individual

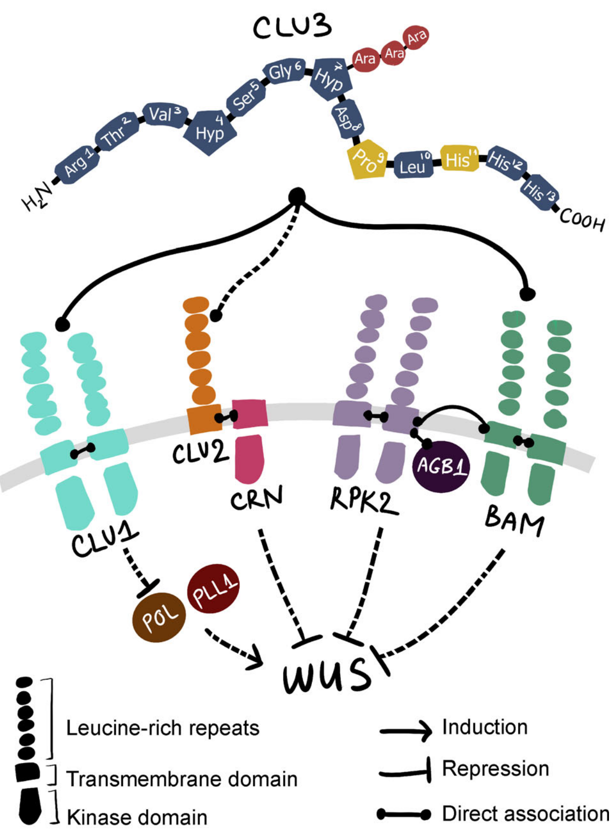

Figure 3 CLV3 signaling pathways. The mature CLV3 peptide forms a horseshoe-shaped kink around the Gly^6 and Hyp^7 residues that is likely recognized by its receptors. The Hyp^7 residue is post-translationally modified with three L-arabinose residues (red). The Pro^9 and His^{11} residues (gold) are most critical for the ability of CLV3 to restrict SAM size in vitro. CLV3 signaling is mediated by a suite of receptors. The LRR-RLK CLV1 forms homodimers that bind to CLV3 peptide, and the protein phosphatases POL and PLL1 act downstream of CLV1 to promote *WUS* transcription. The LRR protein CLV2 and the pseudokinase CRN form a heterodimeric complex that is involved in CLV3 signal transduction, but whether it directly binds CLV3 is unclear. The LRR-RLK RPK2 forms homodimers that associate with the G protein subunit AGB1, but also does not bind directly to CLV3. RPK2 physically interacts with the CLV3-binding LRR-RLK BAM1 in the peripheral zone of the SAM, but not with CLV1 or CLV2. Molecules are not drawn to scale.

residues within the CLV3 peptide to its function in restricting meristem cell accumulation. The transformation of constructs encoding Alanine-substituted CLV3 peptides into *clv3* null mutants revealed that, in order of importance, the Asp^8, His^{11}, Gly^6, Hyp^4, Arg^1 and Pro^9 residues are the most critical for CLV3 activity in the SAM (Song et al., 2012). However, the hydroxyproline residue at position 7 has a minimal impact on CLV3 function, as do the flanking sequences outside the core CLE motif. A follow up study applying synthetic CLV3 peptides to cultured *clv3* null mutant seedlings demonstrated that the Pro^9 and His^{11} residues of the CLV3 peptide (Fig. 3) are the most critical for restricting SAM size *in vitro* (Song et al., 2013). The presence of these two residues positively correlates with CLV3 protein stability *in vitro*, suggesting that CLV3 stability may be important for its role in SAM maintenance.

Sugar modification of the CLV3 peptide is also important for its activity in SAM maintenance. A 13 amino acid hydroxylated and arabinosylated secreted peptide was biochemically identified from *Arabidopsis CLV3* overexpressing plants (Ohyama et al., 2009), in which the Hyp^7 residue of CLV3 is post-translationally modified with three L-arabinose residues (Fig. 3). This modification was shown to enhance CLV3 activity in the seedling SAM (Ohyama et al., 2009). NMR spectroscopy revealed that arabinosylation induces a conformational change in the carboxyl-terminal half of the CLV3 peptide and enhances receptor binding affinity (Shinohara and Matsubayashi, 2013). In tomato, three arabinosyltransferase genes, *FASCIATED INFLORESCENCE (FIN), REDUCED RESIDUAL ARABINOSE 3 (RRA3A)* and *FASCIATED AND BRANCHED2 (FAB2),* are implicated in the arabinosylation of CLV3 peptides (Xu et al., 2015). Mutations in any of these genes cause increase inflorescence branching and the formation of fasciated flowers with extra floral organs. *SlCLV3* null mutants generated using the CRISPR-Cas9 genome editing method display a fasciated SAM phenotype and closely resemble *fin* plants. Synthesized arabinosylated SlCLV3 peptides partially rescue the *fin* fasciated phenotype, confirming the importance of CLV3 arabinosylation *in vivo*. A similar effect was obtained using arabinosylated SlCLE9 peptides, implying a role for SlCLE9 in SAM signaling, although this is yet to be confirmed using mutational analysis. Together, the results indicate that the progressive addition of arabinose chains to CLV3, and potentially to related CLE peptides, by a cascade of arabinosyltransferases is required to fully maintain stem cell homeostasis in the SAM.

CLV3 orthologs are also present in a variety of other crop plants, which over the past ten thousand years have undergone intense selection by humans (Kuittinen and Aguadé, 2000; Doebley et al., 2006) for yield traits such as larger and more numerous inflorescences, fruits, and seeds. The *CLV3* locus has been a target of selection during the domestication of several crop species to enhance agricultural yields (Somssich et al., 2016). A naturally occurring mutation in the mustard *(Brassica rapa) CLV3* gene *MULTILOCULAR4 (ML4)* leads to the formation of fruits with four chambers instead of two, which increases seed production (Fan et al., 2014). Likewise, the mild branching and fasciated flower and fruit phenotype of the classical tomato *fasciated (fas)* allele results from a regulatory mutation at the *SlCLV3* locus that reduces the size of the *CLV3* expression domain without affecting peptide function (Xu et al., 2015). These studies suggest that fine-tuning CLV signaling in the SAM by modulating *CLV3* mRNA expression levels and/or peptide activity may also be exploited in other crops to improve productivity.

CLV3 signal perception

Genetic analyses have uncovered a small suite of membrane-associated receptors that mediate CLV3 signaling in shoot and floral meristems (Fig. 3). However, the contributions of the various receptors to CLV3 signal transduction have remained unclear, as has the functional relationships between them and their relative effects on downstream signaling outputs. Several recent studies provide new insights into these questions.

The first receptor gene shown to play a role in SAM stem cell homeostasis was *CLV1*. Loss-of-function mutations in *CLV1* cause progressive shoot and floral meristem enlargement phenotypes that are similar to but weaker than *clv3* phenotypes, and the two genes act in the same genetic pathway (Clark et al., 1993, 1995; Diévart et al., 2003). CLV1 encodes a leucine-rich repeat (LRR) receptor serine/threonine kinase (Fig. 1B) that is produced in shoot and floral meristem cells interior to the *CLV3*-expressing stem cell domain (Clark et al., 1997). CLV1 is localized to the plasma membrane, where it forms homodimers (Bleckmann et al., 2010) and binds the CLV3 ligand (Ogawa et al., 2008). In contrast with other intercellular signaling pathways in plants, CLV3 appears to bind pre-formed receptor complexes at the plasma membrane (Somssich et al., 2015). Ligand binding triggers activation of the CLV1 kinase domain on the cytosolic surface of the cell, which is thought to lead to recruitment of accessory proteins and to result in CLV1 internalization and trafficking to the lytic vacuole for degradation (Nimchuk et al., 2011b). Signaling through the CLV3-CLV1 ligand-receptor pair limits SAM stem cell accumulation by negatively regulating the *WUS* expression domain in the underlying RZ cells (Brand et al., 2000). The CLV1 receptors are sequestered within plasma membrane microdomains following CLV3 perception, attenuating their signaling activity to prevent complete repression of *WUS* transcription and SAM termination (Somssich et al., 2015).

Genetic and biochemical studies also provide evidence for a second distinct receptor complex involved in CLV3-mediated stem cell signaling, consisting of the CLV2 and CORYNE (CRN) proteins (Guo et al., 2010; Durbak and Tax, 2011). *CLV2* encodes a receptor-like protein with extracellular LRRs, a transmembrane domain and a short cytoplasmic tail (Jeong et al., 1999). Like *CLV3* and *CLV1*, *CLV2* restricts shoot and floral stem cell accumulation (Kayes and Clark, 1998), as does *FASCIATED EAR2 (FEA2)*, the maize ortholog of *CLV2* that maps to a quantitative trait locus (QTL) for kernel row number (Bommert et al., 2013). *CRN* encodes a membrane-associated protein with a cytoplasmic serine/threonine kinase domain, and *crn* mutants display *clv*-like enlarged SAM phenotypes (Müller et al., 2008). Unlike *CLV1*, both *CLV2* and *CRN* are widely expressed in many plant tissues and have broad effects on plant development (Jeong et al., 1999; Müller et al., 2008).

CLV2 and CRN proteins localize to the plasma membrane and form heterodimers (Bleckmann et al., 2010; Zhu et al., 2010). However, CRN lacks kinase activity and is likely to be a pseudokinase that functions as a CLV2 co-receptor (Nimchuk et al., 2011a). Overexpression of *CLV3* in *clv2* plants fails to rescue the enlarged SAM phenotype, indicating that CLV2 is involved in CLV3 signal transduction (Brand et al., 2000). However, the observation that *clv1* phenotypes are enhanced by mutations in either *CLV2* or *CRN* shows that the CLV2-CRN complex functions independently of CLV1 in this process (Müller et al., 2008; Zhu et al., 2010). Whether the CLV2-CRN complex binds CLV3 peptide is unresolved, though, as immunoprecipitation experiments indicate that CLV2 generates a CLV3 binding activity in tobacco leaves (Guo et al., 2010), whereas photo affinity labeling experiments show that CLV2 does not directly bind to arabinosylated CLV3 peptide (Shinohara and Matsubayashi, 2015).

The transcriptional regulation of *CRN* is important for SAM maintenance (Yue et al., 2013). *CRN* transcription is directly repressed by SKB1/PRMT5, a member of the type II arginine methyltransferase family that in animals regulate chromatin remodeling, transcription and pre-mRNA splicing (Bedford and Clarke, 2009). SKB1 directs symmetric dimethylation of histone H4R3 at the *CRN* locus, which leads to upregulation of *CLV3* and *WUS* transcription in their native domains and maintenance of proper SAM size (Yue et al., 2013).

CLV1 forms a monophyletic group with three other LRR-RLK genes, *BARELY ANY MERISTEM1, 2* and *3 (BAM1-3)*, which are predominantly expressed on the flanks of the SAM (DeYoung et al., 2006). Plants carrying higher order combinations of *bam* alleles have reduced SAM size, indicating that the *BAM* genes redundantly promote stem cell maintenance (DeYoung et al., 2006). However, *clv1* null mutant phenotypes can be enhanced by mutations in *BAM1* or *BAM2* (DeYoung and Clark, 2008); thus BAM1 and BAM2 also function as redundant CLV3 receptors in the PZ. Indeed, both BAM1 and BAM2 bind CLV3 peptide (Guo et al., 2010; Shinohara and Matsubayashi, 2015). Because *clv1 bam1* plants are insensitive to exogenous arabinosylated CLV3 peptide treatment, CLV1 and BAM1 activity is sufficient to regulate CLV3-mediated stem cell homeostasis in the SAM (Shinohara and Matsubayashi, 2015). A recent study has clarified the relationship between *CLV1* and the *BAM* genes in SAM maintenance. CLV1 signaling was found to repress the expression of *BAM1* and *BAM3* in the RZ, such that in *clv1* mutants, ectopic *BAM* expression partially compensates for loss of CLV1 activity (Nimchuk et al., 2015). Interestingly, *clv1 bam123* quadruple mutants have stronger vegetative SAM phenotypes than *clv3* null mutants (Nimchuk et al., 2015), indicating that at least one other ligand that acts partially redundantly with CLV3 in SAM maintenance remains be identified.

The BAM1 protein has been shown to physically associate with a LRR receptor-like kinase encoded by the *RECEPTOR-LIKE PROTEIN KINASE2 (RPK2)* gene (Kinoshita et al., 2010). Plants carrying *rpk2* mutations display slightly enlarged SAMs and are insensitive to CLV3 peptide treatment, indicating that RPK2 is involved in CLV3 ligand perception. *RPK2* is expressed uniformly throughout the SAM (Kinoshita et al., 2010), and forms homomers as well as interacting with BAM1. However, it neither associates with CLV1 or CLV2 (Kinoshita et al., 2010; Shimizu et al., 2015) nor binds directly to CLV3 peptide (Shinohara and Matsubayashi, 2015). RPK2 is therefore likely to regulate meristem maintenance by transmitting the CLV3 signal through the BAM1 pathway rather than the CLV1 or CLV2/CRN pathways.

The relationship between CLV1 and the other SAM receptors has been investigated using genetic analysis. Like *CLV1, CLV2* and *CRN* mediate stem cell regulation exclusively in the *WUS*-expressing cells of the RZ (Nimchuk, 2017). However, *CLV2, CRN* and *RPK2* are dispensable for the repression of the *BAM* receptor kinase genes by CLV3-CLV1 signaling. The CLV1-mediated repression of *WUS* transcription and consequent restriction of stem cell accumulation was determined to be genetically separable from its regulation of *BAM* gene expression. CLV1 therefore controls two distinct signaling outputs – the repression of *WUS* transcription and the repression of *BAM* transcription – in SAM stem cell niches in response to the CLV3 ligand independently of the other receptors.

CLV3 signal transduction

Several classes of cytosolic components function in CLV3 signal transduction downstream of ligand binding (Fig. 3). In *Arabidopsis*, the kinase associated protein phosphatase KAPP and a Rho GTPase-related protein physically associate with the cytosolic CLV1 kinase domain (Williams et al., 1997; Trotochaud et al., 1999), while the related protein phosphatase 2C proteins POLTERGEIST (POL) and POL-LIKE1 (PLL1) act downstream of CLV1 to promote stem cell maintenance by regulating *WUS* expression (Song et al., 2006). A mitogen-activated protein kinase (MAPK) activity (Betsuyaku et al., 2011) and a E3 ubiquitin ligase called PLANT U-BOX4 (PUB4) (Kinoshita et al., 2015) have also been implicated in signaling downstream of the CLV receptors, although their roles in the signaling network remain to be precisely defined.

In maize, mutations in the *COMPACT PLANT2 (CT2)* gene, which encodes the alpha-subunit of a heterotrimeric GTP binding protein, cause *clv*-like SAM phenotypes (Bommert et al., 2013). Heterotrimeric GTP binding proteins, which are composed of alpha, beta and gamma subunits, are signaling molecules that link extracellular signals to intracellular readouts (Urano and Jones, 2014). The CT2 protein localizes to the plasma membrane and physically interacts with the FEA2 receptor protein in vitro, suggesting a molecular mechanism through which receptor-like proteins that lack a kinase domain can transmit information inside the cell (Bommert et al., 2013). Similarly, mutations in the *Arabidopsis* G protein beta-subunit1 gene *AGB1* produce enlarged SAMs similar to *clv* mutant SAMs and *AGB1* acts upstream of *WUS* in stem cell homeostasis (Ishida et al., 2014). AGB1 protein physically associates with RPK2 at the plasma membrane, although not with CLV1 or CLV2. In contrast to the situation in maize, *Arabidopsis* Gα activity does not affect SAM function, although Gγ activity has a minor role in limiting SAM size (Ishida et al., 2014). Together these observations indicate a role for heterotrimeric GTP binding proteins in transducing CLV-dependent signals within the recipient cells.

CLV3-independent signaling pathways in SAM regulation

Members of the *ERECTA (ER)* receptor kinase gene family also influence stem cell homeostasis in the SAM. *ER, ERL1* and *ERL2* act redundantly to restrict vegetative SAM activity (Uchida et al., 2013). The promoters of all three genes are active in the SAM, and *er erl1 erl2* seedlings form enlarged SAMs in which the L1 and L2 cells are wider than normal (Chen et al., 2013). Hormone induction experiments suggest that ER family members regulate stem cell homeostasis in the SAM by buffering its responsiveness to cytokinin, which promotes cell proliferation and stem cell activity (Gordon et al., 2009). The ER pathway negatively regulates *WUS* transcription (Chen et al., 2013), although this occurs independently of the CLV pathway (Mandel et al., 2014). In fact, ER, CLV and a third pathway consisting of class III HOMEODOMAIN-LEUCINE ZIPPER (HD-ZIP III) transcription factors (Prigge et al., 2005) act in parallel to regulate SAM size (Mandel et al., 2016). The three pathways seem to affect different aspects of SAM activity, as *CLV3* preferentially restricts SAM cell accumulation along the longitudinal axis whereas *ER* and the *HD-ZIP III* genes restrict its growth along distinct lateral orientations. RNA-seq analysis provides evidence that the CLV pathway limits the accumulation of stem cells in the CZ, whereas the ER pathway regulates mitotic activity in the PZ (Mandel et al., 2016). Thus the coordination of cell behaviors within the SAM appears to be orchestrated by distinct signaling pathways acting along discrete growth vectors.

A novel CLE ligand-receptor signal transduction pathway that regulates maize shoot apical meristem activity has been revealed by the recent study of the *FASCIATED EAR3 (FEA3)* gene (Je et al., 2016). *FEA3* encodes a LRR receptor-like protein with 12 extracellular LRR motifs, a transmembrane domain and a short cytoplasmic tail. FEA3 functions to limit maize SAM size and suppresses *ZmWUS* expression in cells below the OC. However, FEA3 does not perceive a CLV3 signal. Rather it responds to a CLE peptide encoded by the *FON2-LIKE CLE PROTEIN 1 (ZmFCP1)* gene, which is orthologous to the rice *FCP1* gene. Mutations in *ZmFCP1* cause enlarged SAM phenotypes, and *ZmFCP1* and *FEA3* function in the same genetic pathway. Interestingly, *ZmFCP1* is not expressed in the SAM itself but in the initiating organ primordia on the SAM flanks. The authors propose that a ZmFCP1 signal originating from differentiating cells within organ primordia is perceived by FEA3 in the interior of the SAM where it acts to restrict stem cell proliferation by negatively regulating *ZmWUS* expression in the RZ cells beneath the OC. The *Arabidopsis FEA3* ortholog, *AtFEA3*, also appears to restrict SAM activity and *AtFEA3* RNAi lines are resistant to CLE27 peptide application, although *CLE27* is not the *ZmFCP1* ortholog. Thus stem cell homeostasis in plants is mediated by multiple CLE peptides that originate from different cell types and associate with distinct transmembrane receptors.

WUS-CLV3 stem cell homeostasis feedback loop

The key biologically relevant target of the CLV3 stem cell signaling pathway is the *WUSCHEL (WUS)* gene. WUS is the founding member of the WUSCHEL-LIKE HOMEOBOX (WOX) family of transcription factors that contain a homeodomain superficially resembling that found in animal homeodomain proteins (Mayer et al., 1998) (Fig. 1C). In addition, the protein contains three conserved short sequence motifs at the carboxyl terminus: an acidic domain that may function in transcription activation, a canonical WUS box, and an EAR domain that can mediate transcriptional repression (Ohta et al., 2001). *WUS* expression is restricted to a small set of cells just beneath the stem cells (Mayer et al., 1998), which is called the organizing center (OC) based on its functional correspondence to animal stem cell niches (Fig. 2).

Although *WUS* is dispensable for the establishment of the *Arabidopsis* shoot stem cell reservoir (see below), it is required to sustain stem cell activity in shoot and floral meristems throughout the life of the plant (Laux et al., 1996). WUS promotes stem cell fate in a non-cell autonomous fashion, with the protein moving from the OC into the overlying stem cells where it accumulates at a lower level than in the OC cells themselves (Yadav et al., 2011). This movement occurs via cytoplasmic channels between neighboring cells called plasmodesmata and is essential for SAM maintenance (Daum et al., 2014). WUS protein accumulation in the CZ induces the expression of *CLV3* (Fig. 4), activating its own negative regulator in a dynamic feedback loop that regulates stem cell homeostasis in the SAM (Brand et al., 2000; Schoof et al., 2000).

The regulation of *CLV3* transcription by WUS occurs in a dosage-dependent manner. Lower concentrations of WUS protein activate *CLV3* transcription whereas higher concentrations repress *CLV3* transcription (Perales et al., 2016). WUS protein binds with different affinities to six *cis* elements in the regulatory region of the *CLV3* locus, five of which occur in a module in the 3′ region, and these six elements mediate both the activation and repression of *CLV3* expression. WUS binds the *CLV3 cis* elements as monomers at lower concentrations, and as homodimers at higher concentrations (Perales et al., 2016). Structure-function analysis indicates that reduced WUS protein accumulation in the stem cell reservoir may occur through a combination of potent nuclear export and weak nuclear retention of the protein within these cells, potentially due to a lower affinity for DNA and reduced dimerization activity (Rodriguez et al., 2016). In this manner, lower levels of WUS protein in the nucleus of the stem cells leads to *CLV3* activation, whereas higher levels of WUS protein in the nucleus of OC cells leads to *CLV3* repression (Perales et al., 2016).

WUS-dependent gene regulatory network

WUS is a bi-functional protein that can act as both an activator and a repressor of transcription (Ikeda et al., 2009), and regulates the expression of hundreds of genes in the shoot apical meristem. A genome-wide identification of WUS response genes using *Arabidopsis* ATH1 arrays yielded a total of 675 genes (Busch et al., 2010), including 4 hormone responsive type-A *ARABIDOPSIS RESPONSE REGULATOR (ARR)* genes previously described as WUS targets (Leibfried et al., 2005). Gene ontogeny analysis revealed an over-representation of WUS responsive genes in three categories: regulation of development, metabolic processes, and hormone signaling. The *CLV1* gene was found to be directly repressed by WUS despite their overlapping expression patterns in the interior of the SAM, suggesting that WUS acts to fine-tune *CLV1* transcription rather than acting as a binary switch. WUS also directly represses the transcription of *TPR1* and *TPR2*, members of the *TOPLESS/TOPLESS RELATED (TPL/TPR)* family of transcriptional co-repressor genes that play key roles in embryo patterning and auxin responses (Long et al., 2006; Szemenyei et al., 2008). WUS protein was shown to directly bind two distinct DNA motifs, one of which is a G-Box motif with striking similarity to binding sites for proteins involved in stem cell renewal in animals, the zinc-finger homeodomain transcription factor Zeb-1 (Grooteclaes and Frisch, 2000) and the bHLH-ZIP transcription factor MYC (Blackwell et al., 1990).

A second study using an inducible WUS system also identified over 600 WUS-responsive genes in SAM tissue, among which 49 upregulated and 140 downregulated genes are direct WUS targets (Yadav et al., 2013). The majority of WUS-activated genes are expressed in the CZ and OC of the SAM, whereas the majority of WUS-repressed genes are expressed in the PZ. Among the latter, WUS directly binds to the regulatory regions of key transcription factor genes such as *KANADI1 (KAN1), KAN2, ASYMMETRIC LEAVES2* and *YABBY3* that promote organ identity and cell differentiation. Thus WUS controls stem cell homeostasis in part by repressing the expression of differentiation-inducing transcription factor genes in the central regions of the SAM to prevent premature stem cell differentiation.

An important direct target of WUS repression in the OC is the bHLH transcription factor gene *HECATE1 (HEC1)*

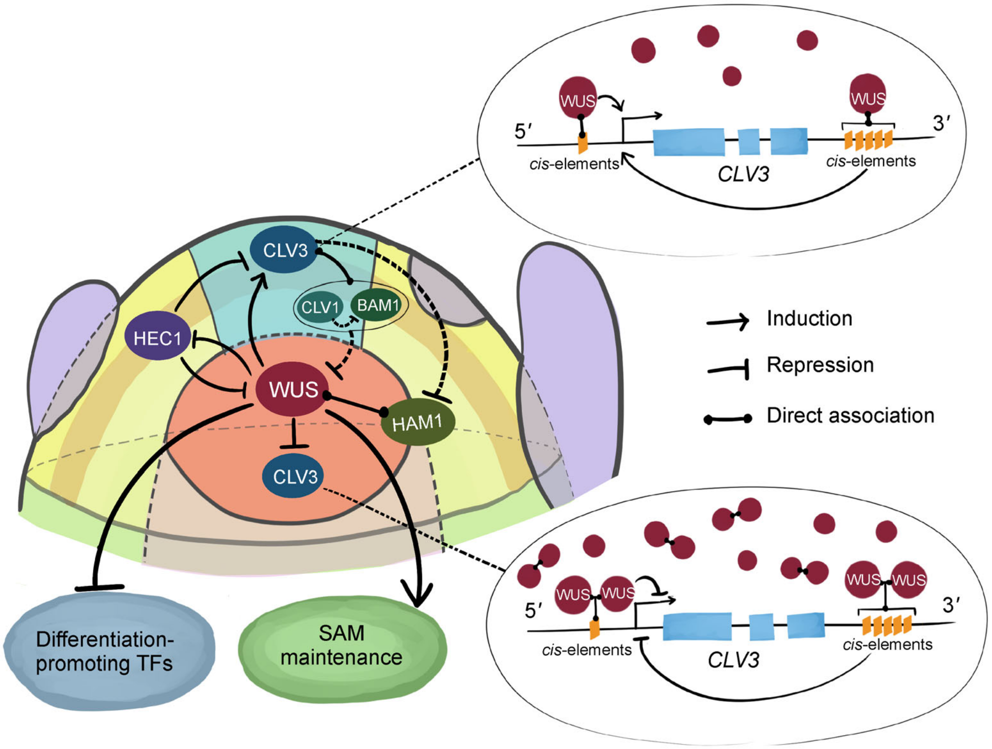

Figure 4 The CLV3-WUS stem cell homeostasis network. CLV3 ligand produced in the CZ diffuses into the underlying OC cells to associate with CLV1 and other receptors. CLV1 binding triggers signaling pathways that repress *BAM1* expression and limit the *WUS* expression domain to the OC. The *WUS* expression domain overlaps with that of the GRAS domain transcriptional regulatory gene *HAM1*, which is repressed by CLV3 signaling, and the WUS and HAM proteins act together as cofactors that share common target genes. WUS activity in the OC represses the expression of differentiation-promoting transcription factors (TFs) and the bHLH TF gene *HEC1*. WUS protein also moves into the adjacent CZ to promote stem cell fate and regulate *CLV3* transcription. WUS protein recognizes cis-elements both upstream and downstream of the *CLV3* coding region (inserts). In the CZ, where WUS protein concentration is low, WUS binds the *cis* elements as a monomer to activate *CLV3* transcription. In the OC, where WUS protein concentration is high, WUS binds the *cis* elements as a dimer to repress *CLV3* transcription. HEC1 activity in the PZ represses *CLV3* and *WUS* expression, and acts oppositely to WUS in the regulation of cytokinin signaling. Together this complex signaling network coordinates stem cell maintenance in the SAM.

(Schuster et al., 2014). *HEC1* is expressed in the PZ of the SAM as well as in developing organ primordia, and functions redundantly with the related *HEC2* and *HEC3* genes to promote SAM cell accumulation. HEC1 activity represses *CLV3* and *WUS* expression while elevating the expression of cell-cycle regulatory genes to stimulate cell proliferation (Fig. 4). In addition, transcriptome analysis indicates that WUS and HEC1 oppositely regulate suites of metabolic and hormone signaling genes, including the type-A *ARR7* and *ARR15* genes. These *ARR* genes are involved in negative feedback regulation of cytokinin signaling (To et al., 2004) and can arrest SAM function when constitutively activated (Leibfried et al., 2005). Whereas WUS directly represses type-A *ARR* gene transcription to enhance cytokinin signaling in the SAM (Leibfried et al., 2005), HEC1 induces their expression and thereby acts as a negative regulator of downstream cytokinin signaling outputs (Schuster et al., 2014). The opposing activities of these two transcription factors in hormone regulation are thought to represent an important mechanism for coordinating a balance between cell proliferation and differentiation in distinct functional domains of the SAM.

WUS-associated factors

WUS does not regulate gene expression in isolation but physically associates with members of the HAIRY MERISTEM (HAM) family of GRAS domain transcriptional regulators (Zhou et al., 2015). Members of this family promote stem cell maintenance in *Arabidopsis* and petunia (Stuurman et al., 2002; Engstrom et al., 2011), and *Arabidopsis ham1234* plants arrest at early seedling stage with terminated SAMs (Zhou et al., 2015). The *HAM* gene expression patterns overlap with that of *WUS* in the SAM, with both *HAM1* and *HAM2* being expressed in the RM, including the OC cells. In addition *HAM1* expression, like *WUS* expression, is repressed by CLV3 signaling. Because a weak *wus-7* allele displays dose-dependent genetic interactions with *ham* null alleles, the strong *wus-1* mutant is epistatic to *ham123* null mutants, and the WUS and HAM proteins share some common downstream regulatory targets (Zhou et al., 2015), the HAM transcriptional regulators are proposed to act as conserved interacting cofactors with WUS in the OC of the SAM (Fig. 4).

WUS also physically associates with TPL and several TPR co-repressor proteins via the acidic domain, WUS box, and EAR motif in the carboxyl-terminal region of the WUS protein (Kieffer et al., 2006; Dolzblasz et al., 2016). The TPL and TPR proteins associate with HISTONE DEACETYLASE19 (HDA19) to form a transcription repression complex (Szemenyei et al., 2008), suggesting a mechanism through which WUS may repress the expression of differentiation-inducing genes within the OC by recruitment of histone modifying complexes. Whether members of either the HAM or TPL/TPR families act together with WUS to regulate *CLV3* transcription remains to be determined.

CLV-WUS activity in early development

The CLV-WUS signal transduction pathway is essential for shoot apical meristem maintenance, but recent studies have revealed unexpected nuances in the pathway during early stem cell initiation and activation. Surprisingly *WUS*, although its expression commences prior to SAM formation at the 16-cell stage of embryogenesis (Mayer et al., 1998), is dispensable for embryonic stem cell initiation and *CLV3* activation (Zhang et al., 2017). Instead the *WUS* paralog *WOX2,* along with the redundant *WOX1, WOX3* and *WOX5* genes, is required for the initiation of the embryonic stem cell program, as assayed by *CLV3* expression. *WOX2* mRNA expression initiates in the zygote and then becomes restricted to the apical lineage (Breuninger et al., 2008). The *WOX2* module downregulates the expression of cotyledon-specific genes and limits the distribution of the synthetic auxin response reporter *DR5:GFP* in apical embryo cells, suggesting that these *WOX* genes block cell differentiation in the presumptive stem cell domain (Zhang et al., 2017). The *WOX2* module also contributes directly to SAM formation by promoting expression in the presumptive SAM region of *HD-ZIP III* genes that induce cytokinin biosynthesis gene transcription and are required for shoot stem cell identity (Prigge et al., 2005; Smith and Long, 2010). The authors propose that the function of the *WOX2* module in embryonic stem cell formation is largely mediated through its upregulation of *HD-ZIP III* gene expression, thereby balancing the activities of the cytokinin and auxin pathways to suppress cell differentiation and promote cell proliferation (Zhang et al., 2017).

Plants undergo distinct post-embryonic developmental programs depending on whether they germinate in the darkness or in the light. In the darkness, the SAM of the germinating seedling remains dormant and growth occurs predominantly via cell elongation in the hypocotyl. Only in the light is the SAM activated and the above ground organ development program triggered. Recent evidence indicates that SAM stem cell identity, as monitored by *CLV3* expression, is sustained even in the dormant state, independent of growth conditions (Pfeiffer et al., 2016). In contrast, *WUS* expression is not detectable in dark-grown seedlings and its induction depends on both light and sucrose, which acts as an energy source for stem cell activity. Shoot stem cell activation by sugars is dependent on a TARGET OF RAPAMYCIN (TOR) kinase-dependent signaling pathway (Dobrenel et al., 2016; Pfeiffer et al., 2016). Light activation of *WUS* expression also occurs via TOR kinase-dependent signaling, independent of photosynthesis, and thus TOR acts as a central regulator of post-embryonic stem cell activation in response to environmental cues. The findings suggest that TOR activation by light enables plant cells to anticipate the amount of energy available for photomorphogenesis and efficiently adapt their growth and development to the local environmental conditions (Pfeiffer et al., 2016).

CLE peptide function during shoot vascular development

Several *CLE* genes also play an important role in vascular development in shoot tissues. The wedge-shaped vascular bundles of the plant stem consist of two conducting tissues, the phloem and the xylem (Fig. 5). The phloem lies laterally and contains sieve tubes that transport sugars and amino acids, whereas the vessels and tracheids of the interior xylem transport water and ions absorbed from the soil. Between these two mature tissues lies a narrow strip of meristematic cells called the procambium or vascular cambium. Procambial cells divide parallel to the plane of the stem to generate phloem cells in one direction and xylem cells in the other direction. This secondary growth property of plant stems allows them to grow radially over long periods of time and is regulated by both systemic hormone signals and localized CLE peptide activity.

TDIF regulates the organization of the stem vascular bundle

TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR (TDIF) is an active peptide derived from the *CLE41* and *CLE44* coding sequences that has two related functions: to promote the proliferation of *Arabidopsis* procambial cells and to inhibit their differentiation into xylem (Ito et al., 2006). The effect of TDIF peptide application to procambial cell proliferation is enhanced by simultaneous treatment with CLE6 peptide (Whitford et al., 2008), although the biological significance of this is as yet unknown. The TDIF ligand is bound by the LRR receptor-like kinase PHLOEM INTERCALATED WITH XYLEM (PXY) (Hirakawa et al., 2008; Etchells and Turner, 2010), also known as TDIF RECEPTOR (TDF), which controls the rate and orientation of procambial cell division (Fisher and Turner, 2007; Etchells and Turner, 2010; Hirakawa et al., 2010). The CLE42 peptide, which differs from TDIF in one amino acid, also has partial TDIF activity (Hirakawa et al., 2008) and shows a weak interaction with the PXY extracellular domain in *in vitro* assays (Zhang et al., 2016). *PXY* also interacts genetically with the *ER* receptor kinase

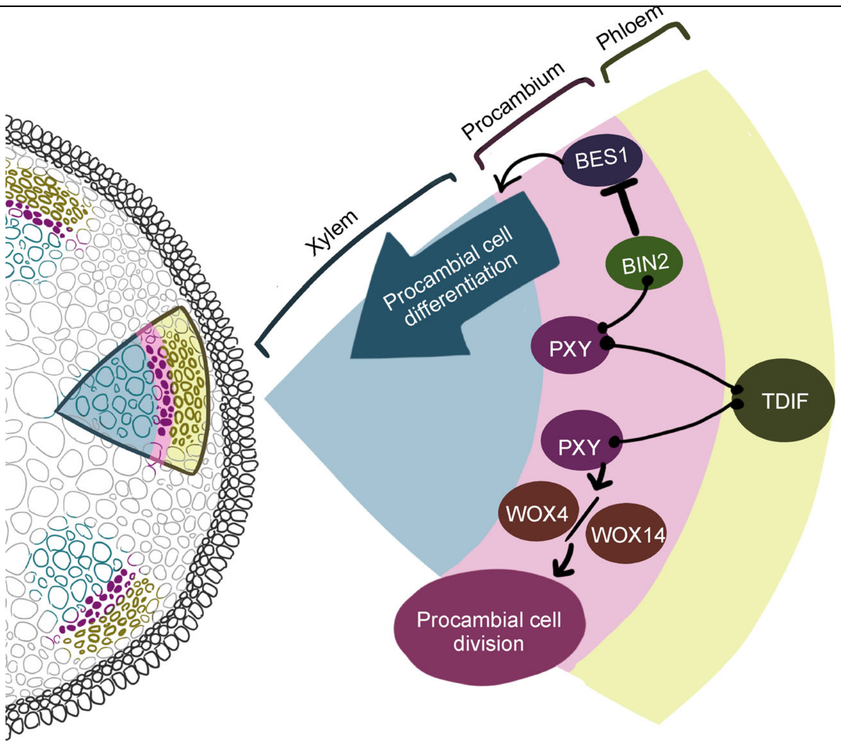

Figure 5 CLE regulation of vascular cambium activity. A wedge-shaped vascular bundle in the *Arabidopsis* stem containing meristematic procambial cells that divide to generate phloem and xylem cells is shown. *CLE41* and *CLE44*, both encoding the same peptide, are expressed in the phloem. The resulting ligand, called TDIF, is perceived by PXY, an LRR-RLK in the procambium. TDIF-PXY signaling induces *WOX4* and *WOX14* expression to promote procambial cell division. In addition, PXY physically interacts with BIN2 to inhibit the transcription factor BES1, thus preventing procambial cell differentiation into xylem.

gene to regulate vascular organization, acting to prevent the intercalation of phloem and xylem in the inflorescence stem vascular bundles (Etchells et al., 2013).

Like its counterparts in the shoot apical meristem, the TDIF/PXY ligand-receptor duo appears to act as a short-range signaling module. *CLE41* and *CLE44* are both expressed in the phloem (Hirakawa et al., 2008; Etchells and Turner, 2010), and TDIF protein can be detected in the extracellular space around phloem precursor cells (Hirakawa et al., 2008). In contrast, *PXY* is specifically expressed in the adjacent vascular procambium (Fisher and Turner, 2007). Thus TDIF signals in a non-cell autonomous fashion from phloem cells to induce the proliferation and suppress the differentiation of the neighboring procambial cells. At the molecular level CLE41 signaling negatively regulates *PXY* expression in inflorescence stems (Etchells and Turner, 2010). Such ligand-mediated repression of receptor gene expression also occurs in animal systems to shape the gradient of ligand activity across tissues (Cadigan et al., 1998).

Several groups have recently reported the crystal structure of the TDIF-PXY ligand-receptor complex (Morita et al., 2016; Zhang et al., 2016; Li et al., 2017). The TDIF peptide consists of 12 amino acids, including two hydroxyproline (Hyp) residues (Ito et al., 2006), whereas PXY belongs to the class XI subfamily of LRR-RLKs and is composed of an extracellular LRR domain, a transmembrane domain and a cytoplasmic kinase domain. Resolution of the PXY extracellular domain crystal structure revealed that it forms a twisted superhelix consisting of 22 LRR motifs, with N-terminal and C-terminal capped ends that are involved in stabilizing the structure (Morita et al., 2016; Zhang et al., 2016). TDIF was found to adopt an extended confirmation that fits in a shallow groove along the LRR4-LRR15 motifs on the inner concave surface of the PXY extracellular domain. A horseshoe-shaped kink around the Gly^6-Hyp^7 residues in the middle of the peptide is recognized by a complimentarily shaped pocket formed by residues in LRR8, LRR9 and LRR11 and is critical for the TDIF-PXY interaction.

The crystal structures provide important insights into the mechanisms of CLE ligand-receptor binding. The kink-forming residues of TDIF are highly conserved among the CLE family peptides, and the Gly^6 residue is required for the binding of TDIF to PXY (Li et al., 2017). Mutations in Gly^6 reduce the activity of TDIF as well as several other CLE peptides, including CLV3 and CLE3 (Fletcher et al., 1999; Ito et al., 2006). Further, the residues in the kink recognition pocket of PXY are conserved among CLE receptors such as CLV1 and BAM1/2/3 (Morita et al., 2016; Li et al., 2017), suggesting that this interface may function as a feature for general recognition of CLE ligands by their receptors. In contrast, the His^1 and Asn^{12} residues at the ends of the peptide appear to be important for the discrimination of TDIF from other CLE molecules by the PXY receptor (Morita et al., 2016; Li et al., 2017).

Downstream components of TDIF signaling

The dual functions of TDIF-PXY signaling in shoot vascular development are mediated by distinct downstream components. A key downstream target of TDIF-PXY signaling to direct procambial cell proliferation is *WOX4*, which is expressed in the vascular procambium and cambium (Hirakawa et al., 2010; Etchells et al., 2013). *WOX4* is rapidly induced by exogenous application of TDIF in a PXY-dependent fashion (Hirakawa et al., 2010), and promotes procambial cell division (Ji et al., 2010; Suer et al., 2011). The *WOX14* gene acts redundantly with *WOX4* to promote procambial cell division but not vascular organization (Etchells et al., 2013). This along with the data that the PXY-ER genetic interaction affects vascular organization but not vascular cell division indicates that these are genetically separable processes that may be regulated by CLE-WOX signaling modules with some shared and some unique constituents.

WOX4 is not required, however, for the suppression of xylem differentiation by TDIF (Hirakawa et al., 2010). Instead, at the plasma membrane of procambial cells, the PXY receptor kinase physically associates with and promotes the kinase activity of BRASSINOSTEROID-INSENSITIVE 2 (BIN2) and other members of the GLYCOGEN SYNTHASE KINASE 3 (GSK3) family of proteins in a TDIF-dependent manner (Kondo et al., 2014). The GSK3 proteins, which also function in brassinosteroid signaling, act redundantly to inhibit procambial cell differentiation into xylem by repressing the activity of the transcription factor BES1. Given that brassinosteroids also promote xylem cell differentiation (Caño-Delgado et al., 2004; Yamamoto et al., 2007), further studies should uncover the extent of crosstalk between the different signal transduction pathways.

Finally, the role of the TDIF/PXY pathway in shoot vascular development in trees, which produce wood via the differentiation of procambium cells into xylem, has been investigated by cloning *PtCLE41* and *PtPXY* from hybrid aspen (Etchells et al., 2015). Molecular complementation experiments showed that both *PtCLE41* and *PtPXY* are functional orthologs of the corresponding *Arabidopsis* genes. Tissue-specific overexpression of *PtCLE41* and *PtPXY* in hybrid aspen produced taller trees with a twofold increase in the rate of wood formation and increased overall woody biomass, indicating that the CLE41 signaling pathway functions to regulate secondary growth in trees by controlling procambial activity. Such knowledge may be exploited to enhance secondary growth and wood formation in commercially grown tree species.

Perspectives and frontiers

Functional analysis of CLE peptide ligands will become increasingly important as systematic genome-wide analyses continue to identify *CLE* gene families in agriculturally valuable crop species. Recently, 84 *CLE* peptide-encoding genes were identified in soybean *(Glycine max)* and 44 in common bean *(Phaseolus vulgaris)* (Hastwell et al., 2015). Phylogenetic analyses of the soybean, common bean and *Arabidopsis* pre-propeptide sequences yielded seven distinct groups based on their CLE domain sequence and predicted function, enabling the distinguishing of soybean and common bean orthologs of the *Arabidopsis* CLV3, CLE40 and the TDIF peptides. In poplar *(Populus trichocarpa),* genome-wide analysis identified a total of 50 *CLE* genes (Han et al., 2016), adding 24 genes to those found in a previous study (Oelkers et al., 2008). The first systematic analysis of *CLE* genes in gymnosperms identified 93 *CLE* genes among eight conifer species (Strabala et al., 2014). In this case, only the TDIF peptide sequence was completely conserved between gymnosperms and angiosperms. Two TDIF orthologs from *Pinus radiata* were shown to be expressed in the root and in the phloem of the inflorescence stem, suggesting a possible conserved role for TDIF peptides in regulating vascular cambium development between dicots and conifers whose ancestors diverged over 270 million years ago (Bowe et al., 2000).

In addition, a phenetic method was used to identify 1628 *CLE* genes from 57 different plant genomes (Goad et al., 2016). This study found two additional *CLE* genes in soybean, two in poplar, and 19 more in maize *(Zea mays)* than previously reported (Je et al., 2016). Up to nine *CLE* genes were identified in mosses and lycophytes, but none were detected in green algae. Clustering analysis based on the full pre-propeptide sequences generated 12 groups of CLE proteins sequences, with CLE peptides known to be involved in meristem activity, vascular development or nodulation clustering together. However, these studies underscore how little information is available about the biological functions of the vast majority of CLE peptide ligands.

The advent of genome engineering through the CRISPR-Cas9 system (Feng et al., 2013; Nekrasov et al., 2013) has the potential to dramatically accelerate our understanding of *CLE* gene function in plants. Unlike other methods such as TILLING (McCallum et al., 2000) or transposon mutagenesis, the small size of the *CLE* coding sequences is not an impediment to generating null mutations using the CRISPR-Cas9 system. Moreover, multiple *CLE* genes that show tight genetic linkage and/or strongly overlapping expression patterns can be targeted simultaneously, helping to surmount the widespread functional redundancy that occurs among *CLE* family members (Jun et al., 2010). The application of genome editing to *CLE* genes in both model plants and crop systems will provide valuable new insights into the mechanisms of cell-to-cell communication in plants as well as an expanded toolkit for augmenting crop plant growth and resilience in response to global climate change.

Compliance with ethics guidelines

Thai Q. Dao and Jennifer C. Fletcher declare that they have no conflict of interest.

References

Bedford M T, Clarke S G (2009). Protein arginine methylation in mammals: who, what, and why. Mol Cell, 33(1): 1–13

Bergeron J J M, Di Guglielmo G M, Dahan S, Dominguez M, Posner B I (2016). Spatial and temporal regulation of receptor tyrosine kinase activation and intracellular signal transduction. Annu Rev Biochem, 85(1): 573–597

Betsuyaku S, Takahashi F, Kinoshita A, Miwa H, Shinozaki K, Fukuda H, Sawa S (2011). Mitogen-activated protein kinase regulated by the CLAVATA receptors contributes to shoot apical meristem homeostasis. Plant Cell Physiol, 52(1): 14–29

Blackwell T K, Kretzner L, Blackwood E M, Eisenman R N, Weintraub H (1990). Sequence-specific DNA binding by the c-Myc protein. Science, 250(4984): 1149–1151

Bleckmann A, Weidtkamp-Peters S, Seidel C A M, Simon R (2010). Stem cell signaling in *Arabidopsis* requires CRN to localize CLV2 to the plasma membrane. Plant Physiol, 152(1): 166–176

Bommert P, Nagasawa N S, Jackson D (2013). Quantitative variation in maize kernel row number is controlled by the FASCIATED EAR2 locus. Nat Genet, 45(3): 334–337

Bowe L M, Coat G, dePamphilis C W (2000). Phylogeny of seed plants based on all three genomic compartments: extant gymnosperms are monophyletic and Gnetales' closest relatives are conifers. Proc Natl Acad Sci USA, 97(8): 4092–4097

Brand U, Fletcher J C, Hobe M, Meyerowitz E M, Simon R (2000). Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by CLV3 activity. Science, 289(5479): 617–619

Breuninger H, Rikirsch E, Hermann M, Ueda M, Laux T (2008). Differential expression of *WOX* genes mediates apical-basal axis formation in the *Arabidopsis* embryo. Dev Cell, 14(6): 867–876

Busch W, Miotk A, Ariel F D, Zhao Z, Forner J, Daum G, Suzaki T, Schuster C, Schultheiss S J, Leibfried A, Haubeiss S, Ha N, Chan R L, Lohmann J U (2010). Transcriptional control of a plant stem cell niche. Dev Cell, 18(5): 849–861

Cadigan K M, Fish M P, Rulifson E J, Nusse R (1998). Wingless repression of *Drosophila* frizzled 2 expression shapes the Wingless morphogen gradient in the wing. Cell, 93(5): 767–777

Caño-Delgado A, Yin Y, Yu C, Vafeados D, Mora-García S, Cheng J C, Nam K H, Li J, Chory J (2004). BRL1 and BRL3 are novel brassinosteroid receptors that function in vascular differentiation in *Arabidopsis*. Development, 131(21): 5341–5351

Casamitjana-Martínez E, Hofhuis H F, Xu J, Liu C M, Heidstra R, 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(16): 1435–1441

Chen M K, Wilson R L, Palme K, Ditengou F A, Shpak E D (2013). ERECTA family genes regulate auxin transport in the shoot apical meristem and forming leaf primordia. Plant Physiol, 162(4): 1978–1991

Clark S E, Running M P, Meyerowitz E M (1993). CLAVATA1, a regulator of meristem and flower development in *Arabidopsis*. Development, 119(2): 397–418

Clark S E, Running M P, 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

Clark S E, Williams R W, Meyerowitz E M (1997). The *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis*. Cell, 89(4): 575–585

Cock J M, McCormick S (2001). A large family of genes that share homology with *CLAVATA3*. Plant Physiol, 126(3): 939–942

Daum G, Medzihradszky A, Suzaki T, Lohmann J U (2014). A mechanistic framework for noncell autonomous stem cell induction in *Arabidopsis*. Proc Natl Acad Sci USA, 111(40): 14619–14624

DeYoung B J, Bickle K L, Schrage K J, Muskett P, Patel K, Clark S E (2006). The CLAVATA1-related BAM1, BAM2 and BAM3 receptor kinase-like proteins are required for meristem function in *Arabidopsis.* Plant J, 45(1): 1–16

DeYoung B J, Clark S E (2008). BAM receptors regulate stem cell specification and organ development through complex interactions with CLAVATA signaling. Genetics, 180(2): 895–904

Diévart A, Dalal M, Tax F E, Lacey A D, Huttly A, Li J, Clark S E (2003). CLAVATA1 dominant-negative alleles reveal functional overlap between multiple receptor kinases that regulate meristem and organ development. Plant Cell, 15(5): 1198–1211

Dobrenel T, Caldana C, Hanson J, Robaglia C, Vincentz M, Veit B, Meyer C (2016). Tor signaling and nutrient sensing. Ann Rev Plant Biol, 67 (1): 261

Doebley J F, Gaut B S, Smith B D (2006). The molecular genetics of crop domestication. Cell, 127(7): 1309–1321

Dolzblasz A, Nardmann J, Clerici E, Causier B, van der Graaff E, Chen J, Davies B, Werr W, Laux T (2016). Stem cell regulation by *Arabidopsis WOX* genes. Mol Plant, 9(7): 1028–1039

Durbak A R, Tax F E (2011). CLAVATA signaling pathway receptors of *Arabidopsis* regulate cell proliferation in fruit organ formation as well as in meristems. Genetics, 189(1): 177–194

Engstrom E M, Andersen C M, Gumulak-Smith J, Hu J, Orlova E, Sozzani R, Bowman J L (2011). *Arabidopsis* homologs of the *petunia HAIRY MERISTEM* gene are required for maintenance of shoot and root indeterminacy. Plant Physiol, 155(2): 735–750

Etchells J P, Mishra L S, Kumar M, Campbell L, Turner S R (2015). Wood formation in trees is increased by manipulating PXY-regulated cell division. Curr Biol, 25(8): 1050–1055

Etchells J P, Provost C M, Mishra L, Turner S R (2013). WOX4 and WOX14 act downstream of the PXY receptor kinase to regulate plant vascular proliferation independently of any role in vascular organisation. Development, 140(10): 2224–2234

Etchells J P, Turner S R (2010). The PXY-CLE41 receptor ligand pair defines a multifunctional pathway that controls the rate and orientation of vascular cell division. Development, 137(5): 767–774

Fan C, Wu Y, Yang Q, Yang Y, Meng Q, Zhang K, Li J, Wang J, Zhou Y (2014). A novel single-nucleotide mutation in a *CLAVATA3* gene homolog controls a multilocular silique trait in *Brassica rapa* L. Mol Plant, 7(12): 1788–1792

Feng Z, Zhang B, Ding W, Liu X, Yang D L, Wei P, Cao F, Zhu S, Zhang F, Mao Y, Zhu J K (2013). Efficient genome editing in plants using a CRISPR/Cas system. Cell Res, 23(10): 1229–1232

Fisher K, Turner S (2007). PXY, a receptor-like kinase essential for maintaining polarity during plant vascular-tissue development. Curr Biol, 17(12): 1061–1066

Fletcher J C, Brand U, Running M P, Simon R, Meyerowitz E M (1999). Signaling of cell fate decisions by CLAVATA3 in *Arabidopsis* shoot meristems. Science, 283(5409): 1911–1914

Furner I J, Pumfrey J E (1992). Cell fate in the shoot apical meristem of *Arabidopsis thaliana*. Development, 115: 755–764

Gifford E M (1954). The shoot apex in angiosperms. Bot Rev, 20(8): 429–447

Goad D M, Zhu C, Kellogg E A (2017). Comprehensive identification and clustering of CLV3/ESR-related (CLE) genes in plants finds groups with potentially shared function. New Phytol, 216(2):605–616

Gordon S P, Chickarmane V S, Ohno C, Meyerowitz E M (2009). Multiple feedback loops through cytokinin signaling control stem cell number within the *Arabidopsis* shoot meristem. Proc Natl Acad Sci USA, 106(38): 16529–16534

Grooteclaes M L, Frisch S M (2000). Evidence for a function of CtBP in epithelial gene regulation and anoikis. Oncogene, 19(33): 3823–3828

Guo Y, Han L, Hymes M, Denver R, Clark S E (2010). CLAVATA2 forms a distinct CLE-binding receptor complex regulating *Arabidopsis* stem cell specification. Plant J, 63(6): 889–900

Han H, Zhang G, Wu M, Wang G (2016). Identification and characterization of the *Populus trichocarpa* CLE family. BMC Genomics, 17(1): 174

Hastwell A H, Gresshoff P M, Ferguson B J (2015). Genome-wide annotation and characterization of CLAVATA/ESR (CLE) peptide hormones of soybean *(Glycine max)* and common bean (*Phaseolus vulgaris*), and their orthologues of *Arabidopsis thaliana.* J Exp Bot, 66(17): 5271–5287

Hirakawa Y, Kondo Y, Fukuda H (2010). TDIF peptide signaling regulates vascular stem cell proliferation via the *WOX4* homeobox gene in *Arabidopsis.* Plant Cell, 22(8): 2618–2629

Hirakawa Y, Shinohara H, Kondo Y, Inoue A, Nakanomyo I, Ogawa M, Sawa S, Ohashi-Ito K, Matsubayashi Y, Fukuda H (2008). Non-cell-autonomous control of vascular stem cell fate by a CLE peptide/receptor system. Proc Natl Acad Sci USA, 105(39): 15208–15213

Ikeda M, Mitsuda N, Ohme-Takagi M (2009). *Arabidopsis* WUSCHEL is a bifunctional transcription factor that acts as a repressor in stem cell regulation and as an activator in floral patterning. Plant Cell, 21 (11): 3493–3505

Irish V F, Sussex I M (1992). A fate map of the *Arabidopsis* embryonic shoot apical meristem. Development, 115: 745–753

Ishida T, Tabata R, Yamada M, Aida M, Mitsumasu K, Fujiwara M, Yamaguchi K, Shigenobu S, Higuchi M, Tsuji H, Shimamoto K, Hasebe M, Fukuda H, Sawa S (2014). Heterotrimeric G proteins control stem cell proliferation through CLAVATA signaling in *Arabidopsis.* EMBO Rep, 15(11): 1202–1209

Ito Y, Nakanomyo I, Motose H, Iwamoto K, Sawa S, Dohmae N, Fukuda H (2006). Dodeca-CLE peptides as suppressors of plant stem cell differentiation. Science, 313(5788): 842–845

Je B I, Gruel J, Lee Y K, Bommert P, Arevalo E D, Eveland A L, Wu Q, Goldshmidt A, Meeley R, Bartlett M, Komatsu M, Sakai H, Jönsson H, Jackson D (2016). Signaling from maize organ primordia via FASCIATED EAR3 regulates stem cell proliferation and yield traits. Nat Genet, 48(7): 785–791

Jeong S, Trotochaud A E, 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(10): 1925–1934

Ji J, Strable J, Shimizu R, Koenig D, Sinha N, Scanlon M J (2010). WOX4 promotes procambial development. Plant Physiol, 152(3): 1346–1356

Jun J, Fiume E, Roeder A H K, Meng L, Sharma V K, Osmont K S, Baker C, Ha C M, Meyerowitz E M, Feldman L J, Fletcher J C (2010). Comprehensive analysis of CLE polypeptide signaling gene expression and overexpression activity in *Arabidopsis.* Plant Physiol, 154(4): 1721–1736

Kayes J M, Clark S E (1998). CLAVATA2, a regulator of meristem and organ development in *Arabidopsis*. Development, 125(19): 3843–3851

Kieffer M, Stern Y, Cook H, Clerici E, Maulbetsch C, Laux T, Davies B (2006). Analysis of the transcription factor WUSCHEL and its functional homologue in *Antirrhinum* reveals a potential mechanism for their roles in meristem maintenance. Plant Cell, 18(3): 560–573

Kinoshita A, Betsuyaku S, Osakabe Y, Mizuno S, Nagawa S, Stahl Y, Simon R, Yamaguchi-Shinozaki K, Fukuda H, Sawa S (2010). RPK2 is an essential receptor-like kinase that transmits the CLV3 signal in *Arabidopsis*. Development, 137(22): 3911–3920

Kinoshita A, Seo M, Kamiya Y, Sawa S (2015). Mystery in genetics: PUB4 gives a clue to the complex mechanism of CLV signaling pathway in the shoot apical meristem. Plant Signal Behav, 10(6): e1028707

Kondo T, Sawa S, Kinoshita A, Mizuno S, Kakimoto T, Fukuda H, Sakagami Y (2006). A plant peptide encoded by CLV3 identified by in situ MALDI-TOF MS analysis. Science, 313(5788): 845–848

Kondo Y, Ito T, Nakagami H, Hirakawa Y, Saito M, Tamaki T, Shirasu K, Fukuda H (2014). Plant GSK3 proteins regulate xylem cell differentiation downstream of TDIF-TDR signalling. Nat Commun, 5: 3504

Kuittinen H, Aguadé M (2000). Nucleotide variation at the CHALCONE ISOMERASE locus in *Arabidopsis thaliana*. Genetics, 155(2): 863–872

Laux T, Mayer K F X, Berger J, Jürgens G (1996). The WUSCHEL gene is required for shoot and floral meristem integrity in *Arabidopsis*. Development, 122(1): 87–96

Lease K A, Walker J C (2006). The *Arabidopsis* unannotated secreted peptide database, a resource for plant peptidomics. Plant Physiol, 142 (3): 831–838

Leibfried A, To J P C, Busch W, Stehling S, Kehle A, Demar M, Kieber J J, Lohmann J U (2005). WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. Nature, 438(7071): 1172–1175

Li Z, Chakraborty S, Xu G (2017). Differential CLE peptide perception by plant receptors implicated from structural and functional analyses of TDIF-TDR interactions. PLoS One, 12(4): e0175317

Long J A, Ohno C, Smith Z R, Meyerowitz E M (2006). TOPLESS regulates apical embryonic fate in *Arabidopsis*. Science, 312(5779): 1520–1523

Mandel T, Candela H, Landau U, Asis L, Zelinger E, Carles C C, Williams L E (2016). Differential regulation of meristem size, morphology and organization by the ERECTA, CLAVATA and class III HD-ZIP pathways. Development, 143(9): 1612–1622

Mandel T, Moreau F, Kutsher Y, Fletcher J C, Carles C C, Eshed

Williams L (2014). The ERECTA receptor kinase regulates *Arabidopsis* shoot apical meristem size, phyllotaxy and floral meristem identity. Development, 141(4): 830–841

Matsubayashi Y (2014). Posttranslationally modified small-peptide signals in plants. Annu Rev Plant Biol, 65(1): 385–413

Mayer K F X, Schoof H, Haecker A, Lenhard M, Jürgens G, Laux T (1998). Role of WUSCHEL in regulating stem cell fate in the *Arabidopsis* shoot meristem. Cell, 95(6): 805–815

McCallum C M, Comai L, Greene E A, Henikoff S (2000). Targeting Induced Local Lesions IN Genomes (TILLING) for plant functional genomics. Plant Physiol, 123(2): 439–442

Meng L, Ruth K C, Fletcher J C, Feldman L (2010). The roles of different CLE domains in *Arabidopsis* CLE polypeptide activity and functional specificity. Mol Plant, 3(4): 760–772

Morita J, Kato K, Nakane T, Kondo Y, Fukuda H, Nishimasu H, Ishitani R, Nureki O (2016). Crystal structure of the plant receptor-like kinase TDR in complex with the TDIF peptide. Nat Comm, 7:12383

Müller R, Bleckmann A, Simon R (2008). The receptor kinase CORYNE of *Arabidopsis* transmits the stem cell-limiting signal CLAVATA3 independently of CLAVATA1. Plant Cell, 20(4): 934–946

Nekrasov V, Staskawicz B, Weigel D, Jones J D G, Kamoun S (2013). Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. Nat Biotechnol, 31(8): 691–693

Ni J, Clark S E (2006). Evidence for functional conservation, sufficiency, and proteolytic processing of the CLAVATA3 CLE domain. Plant Physiol, 140(2): 726–733

Ni J, Guo Y, Jin H, Hartsell J, Clark S E (2011). Characterization of a CLE processing activity. Plant Mol Biol, 75(1-2): 67–75

Nimchuk Z L (2017). CLAVATA1 controls distinct signaling outputs that buffer shoot stem cell proliferation through a two-step transcriptional compensation loop. PLoS Genet, 13(3): e1006681

Nimchuk Z L, Tarr P T, Meyerowitz E M (2011a). An evolutionarily conserved pseudokinase mediates stem cell production in plants. Plant Cell, 23(3): 851–854

Nimchuk Z L, Tarr P T, Ohno C, Qu X, Meyerowitz E M (2011b). Plant stem cell signaling involves ligand-dependent trafficking of the CLAVATA1 receptor kinase. Curr Biol, 21(5): 345–352

Nimchuk Z L, Zhou Y, Tarr P T, Peterson B A, Meyerowitz E M (2015). Plant stem cell maintenance by transcriptional cross-regulation of related receptor kinases. Development, 142(6): 1043–1049

Oelkers K, Goffard N, Weiller G F, Gresshoff P M, Mathesius U, Frickey T (2008). Bioinformatic analysis of the CLE signaling peptide family. BMC Plant Biol, 8(1): 1

Ogawa M, Shinohara H, Sakagami Y, Matsubayashi Y (2008). *Arabidopsis* CLV3 peptide directly binds CLV1 ectodomain. Science, 319(5861): 294

Ohta M, Matsui K, Hiratsu K, Shinshi H, Ohme-Takagi M (2001). Repression domains of class II ERF transcriptional repressors share an essential motif for active repression. Plant Cell, 13(8): 1959–1968

Ohyama K, Shinohara H, Ogawa-Ohnishi M, Matsubayashi Y (2009). A glycopeptide regulating stem cell fate in *Arabidopsis thaliana.* Nat Chem Biol, 5(8): 578–580

Perales M, Rodriguez K, Snipes S, Yadav R K, Diaz-Mendoza M, Reddy G V (2016). Threshold-dependent transcriptional discrimination underlies stem cell homeostasis. Proc Natl Acad Sci USA, 113(41): E6298–E6306

Pfeiffer A, Janocha D, Dong Y, Medzihradszky A, Schöne S, Daum G, Suzaki T, Forner J, Langenecker T, Rempel E, Schmid M, Wirtz M, Hell R, Lohmann J U (2016). Integration of light and metabolic signals for stem cell activation at the shoot apical meristem. eLife, 5: e17023

Poethig R S (1987). Clonal analysis of cell lineage patterns in plant development. Am J Bot, 74(4): 581–194

Poethig R S, Coe E H J Jr, Johri M M (1986). Cell lineage patterns in maize *Zea mays* embryogenesis: A clonal analysis. Dev Biol, 117(2): 392–404

Poethig R S, Sussex I M (1985a). The cellular parameters of leaf development in tobacco: a clonal analysis. Planta, 165(2): 170–184

Poethig R S, Sussex I M (1985b). The developmental morphology and growth dynamics of the tobacco leaf. Planta, 165(2): 158–169

Prigge M J, Otsuga D, Alonso J M, Ecker J R, Drews G N, Clark S E (2005). Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in *Arabidopsis* development. Plant Cell, 17(1): 61–76

Reddy G V, Meyerowitz E M (2005). Stem-cell homeostasis and growth dynamics can be uncoupled in the *Arabidopsis* shoot apex. Science, 310(5748): 663–667

Rodriguez K, Perales M, Snipes S, Yadav R K, Diaz-Mendoza M, Reddy G V (2016). DNA-dependent homodimerization, sub-cellular partitioning, and protein destabilization control WUSCHEL levels and spatial patterning. Proc Natl Acad Sci USA, 113(41): E6307–E6315

Rojo E, Sharma V K, Kovaleva V, Raikhel N V, Fletcher J C (2002). CLV3 is localized to the extracellular space, where it activates the *Arabidopsis* CLAVATA stem cell signaling pathway. Plant Cell, 14 (5): 969–977

Satina S, Blakeslee A F, Avery A G (1940). Demonstration of the three germ layers in the shoot apex of Datura by means of induced polyploidy in periclinal chimeras. Am J Bot, 27(10): 895–905

Schoof H, Lenhard M, Haecker A, Mayer K F X, Jürgens G, Laux T (2000). The stem cell population of *Arabidopsis* shoot meristems in maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. Cell, 100(6): 635–644

Schuster C, Gaillochet C, Medzihradszky A, Busch W, Daum G, Krebs M, Kehle A, Lohmann J U (2014). A regulatory framework for shoot stem cell control integrating metabolic, transcriptional, and phytohormone signals. Dev Cell, 28(4): 438–449

Sharma V K, Ramirez J, Fletcher J C (2003). The *Arabidopsis* CLV3-like (CLE) genes are expressed in diverse tissues and encode secreted proteins. Plant Mol Biol, 51(3): 415–425

Shimizu N, Ishida T, Yamada M, Shigenobu S, Tabata R, Kinoshita A, Yamaguchi K, Hasebe M, Mitsumasu K, Sawa S (2015). BAM 1 and RECEPTOR-LIKE PROTEIN KINASE 2 constitute a signaling pathway and modulate CLE peptide-triggered growth inhibition in *Arabidopsis* root. New Phytol, 208(4): 1104–1113

Shinohara H, Matsubayashi Y (2013). Chemical synthesis of *Arabidopsis* CLV3 glycopeptide reveals the impact of hydroxyproline arabinosylation on peptide conformation and activity. Plant Cell Physiol, 54(3): 369–374

Shinohara H, Matsubayashi Y (2015). Reevaluation of the CLV3-receptor interaction in the shoot apical meristem: dissection of the CLV3 signaling pathway from a direct ligand-binding point of view. Plant J, 82(2): 328–336

Shiu S H, 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(19): 10763–10768

Smith Z R, Long J A (2010). Control of *Arabidopsis* apical-basal embryo polarity by antagonistic transcription factors. Nature, 464(7287): 423–426

Somssich M, Je B I, Simon R, Jackson D (2016). CLAVATA-WUSCHEL signaling in the shoot meristem. Development, 143 (18): 3238–3248

Somssich M, Ma Q, Weidtkamp-Peters S, Stahl Y, Felekyan S, Bleckmann A, Seidel C A M, Simon R (2015). Real-time dynamics of peptide ligand-dependent receptor complex formation in planta. Sci Signal, 8(388): ra76

Song S K, Lee M M, Clark S E (2006). POL and PLL1 phosphatases are CLAVATA1 signaling intermediates required for *Arabidopsis* shoot and floral stem cells. Development, 133(23): 4691–4698

Song X F, Xu T T, Ren S C, Liu C M (2013). Individual amino acid residues in CLV3 peptide contribute to its stability *in vitro*. Plant Signal Behav, 8(9): 8

Song X F, Yu D L, Xu T T, Ren S C, Guo P, Liu C M (2012). Contributions of individual amino acid residues to the endogenous CLV3 function in shoot apical meristem maintenance in *Arabidopsis.* Mol Plant, 5(2): 515–523

Steeves T A, Sussex I M (1989). Patterns in Plant Development. New York: Cambridge University Press.

Strabala T J, Phillips L, West M, Stanbra L (2014). Bioinformatic and phylogenetic analysis of the *CLAVATA3/EMBRYO-SURROUNDING REGION (CLE)* and the *CLE-LIKE* signal peptide genes in the Pinophyta. BMC Plant Biol, 14(1): 47

Stuurman J, Jäggi F, Kuhlemeier C (2002). Shoot meristem maintenance is controlled by a GRAS-gene mediated signal from differentiating cells. Genes Dev, 16(17): 2213–2218

Suer S, Agusti J, Sanchez P, Schwarz M, Greb T (2011). WOX4 imparts auxin responsiveness to cambium cells in *Arabidopsis*. Plant Cell, 23 (9): 3247–3259

Sussex I M (1954). Experiments on the cause of dorsiventrality in leaves. Nature, 174(4425): 351–352

Szemenyei H, Hannon M, Long J A (2008). TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis. Science, 319(5868): 1384–1386

Tavormina P, De Coninck B, Nikonorova N, De Smet I, Cammue B P (2015). The plant peptidome: an expanding repertoire of structural features and biological functions. Plant Cell, 27(8): 2095–2118

To J P C, Haberer G, Ferreira F J, Deruère J, Mason M G, Schaller G E, Alonso J M, Ecker J R, Kieber J J (2004). Type-A *Arabidopsis* response regulators are partially redundant negative regulators of cytokinin signaling. Plant Cell, 16(3): 658–671

Trotochaud A E, Hao T, Wu G, Yang Z, 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(3): 393–406

Uchida N, Shimada M, Tasaka M (2013). *ERECTA*-family receptor kinases regulate stem cell homeostasis via buffering its cytokinin responsiveness in the shoot apical meristem. Plant Cell Physiol, 54 (3): 343–351

Urano D, Jones A M (2014). Heterotrimeric G protein-coupled signaling in plants. Annu Rev Plant Biol, 65(1): 365–384

Wang X, Mitchum M G, Gao B, Li C, Diab H, Baum T J, Hussey R S, 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(2): 187–191

Whitford R, Fernandez A, De Groodt R, Ortega E, Hilson P (2008). Plant CLE peptides from two distinct functional classes synergistically induce division of vascular cells. Proc Natl Acad Sci USA, 105(47): 18625–18630

Williams R W, Wilson J M, Meyerowitz E M (1997). A possible role for kinase-associated protein phosphatase in the *Arabidopsis* CLAVATA1 signaling pathway. Proc Natl Acad Sci USA, 94(19): 10467–10472

Xu C, Liberatore K L, MacAlister C A, Huang Z, Chu Y H, Jiang K, Brooks C, Ogawa-Ohnishi M, Xiong G, Pauly M, Van Eck J, Matsubayashi Y, van der Knaap E, Lippman Z B (2015). A cascade of arabinosyltransferases controls shoot meristem size in tomato. Nat Genet, 47(7): 784–792

Xu T T, Song X F, Ren S C, Liu C M (2013). The sequence flanking the N-terminus of the CLV3 peptide is critical for its cleavage and activity in stem cell regulation in *Arabidopsis.* BMC Plant Biol, 13 (1): 225

Yadav R K, Perales M, Gruel J, Girke T, Jönsson H, Reddy G V (2011). WUSCHEL protein movement mediates stem cell homeostasis in the *Arabidopsis* shoot apex. Genes Dev, 25(19): 2025–2030

Yadav R K, Perales M, Gruel J, Ohno C, Heisler M, Girke T, Jönsson H, Reddy G V (2013). Plant stem cell maintenance involves direct transcriptional repression of differentiation program. Mol Syst Biol, 9 (1): 654

Yamamoto R, Fujioka S, Iwamoto K, Demura T, Takatsuto S, Yoshida S, Fukuda H (2007). Co-regulation of brassinosteroid biosynthesis-related genes during xylem cell differentiation. Plant Cell Physiol, 48 (1): 74–83

Yue M, Li Q, Zhang Y, Zhao Y, Zhang Z, Bao S (2013). Histone H4R3 methylation catalyzed by SKB1/PRMT5 is required for maintaining shoot apical meristem. PLoS One, 8(12): e83258

Zhang H, Lin X, Han Z, Qu L J, Chai J (2016). Crystal structure of PXY-TDIF complex reveals a conserved recognition mechanism among CLE peptide-receptor pairs. Cell Res, 26(5): 543–555

Zhang Z, Tucker E, Hermann M, Laux T (2017). A molecular framework for the embryonic initiation of shoot meristem stem cells. Dev Cell, 40(3): 264–277.e4

Zhou Y, Liu X, Engstrom E M, Nimchuk Z L, Pruneda-Paz J L, Tarr P T, Yan A, Kay S A, Meyerowitz E M (2015). Control of plant stem cell function by conserved interacting transcriptional regulators. Nature, 517(7534): 377–380

Zhu Y, Wang Y, Li R, Song X, Wang Q, Huang S, Jin J B, Liu C M, Lin J (2010). Analysis of interactions among the CLAVATA3 receptors reveals a direct interaction between CLAVATA2 and CORYNE in *Arabidopsis*. Plant J, 61(2): 223–233