

Peptides Regulating Apical Meristem Development

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Abstract Small signaling peptides are involved in numerous aspects of plant development. A well-studied group of peptides is the CLE family with its most prominent member CLAVATA 3 (CLV3), a key regulator of apical meristem maintenance during growth and development. Members of the CLE peptide family share a high sequence similarity, but are capable of triggering independent pathways. Recent work has revealed complex networks of interacting receptors that recognize different peptides, as well as various posttranslational modifications of the peptides which could account for the signaling specificities within the family. With the CLV pathway well studied in *Arabidopsis*, the insight gained in this model plant can now be transferred to other plant species to control meristem development.

1 Peptides Regulating Apical Meristem Development

The organization, development, and maintenance of multicellular organisms depend on signaling networks that coordinate and integrate cells and tissue responses. Short- and long-range communication between different parts within an organism utilizes direct cell–cell communication, in part based on peptide signaling. In plants, secreted peptides signal via receptors that are mostly in the plasma membrane. The signaling peptides include molecules with diverse biological functions such as systemin, AtPep1, phytosulfokines, and CLAVATA3 (CLV3), a member of the large CLE peptide family. The focus of this chapter is the role of signaling peptides in mediating meristem cell communication and determination of cell fate.

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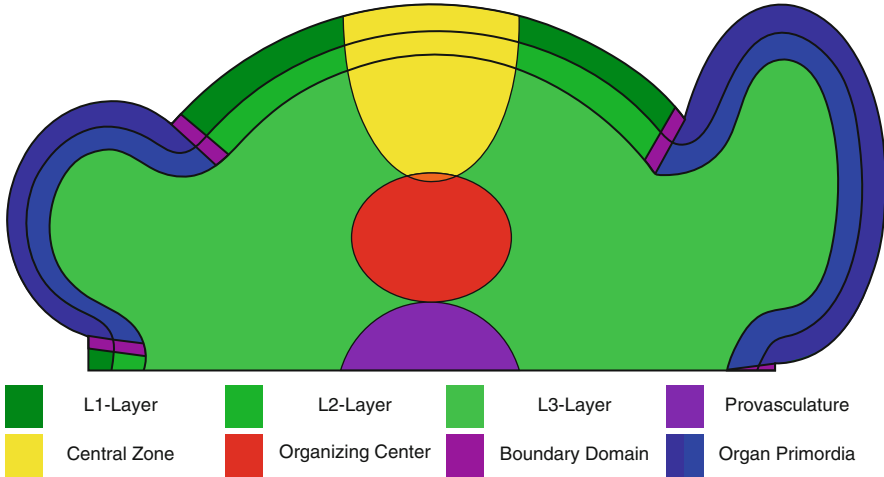


Fig. 1 Functional domains in the shoot apical meristem. Model of the *Arabidopsis* shoot apical meristem. The different subdomains are *color-coded*, based on different functions, gene expression profiles, and cell division rates

2 Structure of the Shoot Apical Meristem

The apical meristems are above-ground stem cell tissues. The main meristem, the shoot apical meristem (SAM), is located at the tip of the shoot and initiates the formation of all above-ground organs and consists of a dome-shaped group of cells that can be divided into specific subdomains based on their functions and gene expression profiles (see Fig. 1). The stem cell population is located at the center of the SAM apex, in the central zone (CZ). Stem cells are undifferentiated and divide slowly. Their daughter cells are displaced toward the peripheral zone (PZ) that encloses the central zone. In the peripheral zone, the cells divide more rapidly and give rise to daughter cells that can enter differentiation pathways or can become part of newly formed organs at the boundary of the meristem. Thus, the peripheral zone serves as a transit-amplifying zone.

The SAM also shows an organization into three distinct layers (L1–L3), which represent individual clones. The outermost layer (L1) contains the cells that will form the epidermis, while the cells of the L2 will form subepidermal tissue and the gametes. Cells in these two layers divide only anticlinally, that is, perpendicular to the meristem surface. All remaining cells form the L3 layer. Their cell divisions are not oriented, and they form the main body of the plant.

The stem cell containing central zone comprises cells of all three layers. Directly underneath these cells is a small population of cells with a low division rate that form the organizing center (OC), which in turn is necessary to initiate and maintain the stem cells of the central zone.

When a plant enters the reproductive stage of development, shoot meristems are transformed into inflorescence meristems (IM) that give rise to a number of lateral floral meristems (FM) that generate the floral organs. Controlled activity of meristems requires communication between stem cells and cells of neighboring tissues.

3 Genes Controlling Stem Cell Activity in the Apical Meristems

In *Arabidopsis thaliana*, the *fully fasciated* (*fuf*) mutants, *fasciata1* and 2 (*fas1/2*), as well as *clavata 1* (*clv1*) were among the first reported mutants that exhibited an enlarged meristem (Leyser and Furner 1992; Medford et al. 1992). Among them, *clv1* was genetically mapped as early as 1983. In 1989, *clv1* was described to have additional inner floral organs, resulting in a club-shaped pistil and silique (*clava* = club, *latin*) (Koornneef et al. 1983; Okada et al. 1989). In subsequent work, *CLV1* was established as a major regulator of SAM and floral meristem development. In addition to the generally enlarged meristems, caused by the accumulation of stem cells in the central zone, the mutants also exhibit more floral organs than the wild type (Clark et al. 1993). *CLV1* encodes a membrane-associated leucine-rich repeat (LRR) receptor-like kinase (RLK) with the LRR receptor domain on the extracellular and a kinase domain on the intracellular side of the membrane. It is first expressed in the shoot meristem during embryogenesis and later both in shoot and floral meristems. Within the meristem, it is expressed in L2 and L3 layer cells of the central zone, as well as cells of the organizing center (OC) (Clark et al. 1997).

In 1998, Kayes et al. described the *clv2* mutant, which resembles a weak *clv1* mutant with respect to the effects on meristem size and organ number. Additionally, *clv2* mutants develop abnormal organs, such as reduced anthers and elongated pedicels, indicating that *CLV2* acts together with *CLV1* in a pathway regulating the meristem, but independently with regard to organ development (Kayes and Clark 1998). *CLV2* encodes a membrane-associated LRR receptor-like protein (RLP), which lacks an intracellular kinase domain. While *CLV2* is prominently expressed in the shoot apex, RNA was detected in all tissues examined (Jeong et al. 1999).

CLV1 and *CLV2* were expected to act together, possibly as a heterodimer, in a pathway regulating stem cell number and overall meristem size. In 2008, Müller et al. identified *CORYNE* (*CRN*), a membrane-associated protein kinase (PK) *crn* mutants are phenotypically weaker than *clv1*, but very similar to *clv2* mutants, also showing stem cell accumulation and organ defects. While *CLV1* represents a full RLK, *CLV2*, which lacks the kinase domain, and *CRN*, which lacks a receptor domain, could complement each other. Because genetic data further revealed additive effects in *clv1/clv2* and *clv1/crn* double mutants, a new model was proposed with *CLV1* functioning in parallel to a complex consisting of *CLV2* and *CRN*. These two distinct pathways may, however, be interconnected through

Table 1 Genes involved in peptide-related meristem maintenance

Name	Annotation	Protein type	Expression in SAM	Proposed function
Peptides				
<i>CLV3</i>	<i>AT2G27250</i>	Peptide	Stem cells	Promotes stem cell differentiation
<i>CLE42</i>	<i>AT2G34925</i>	Peptide	Entire SAM	Could promote stem cell fate
Receptor/kinases				
<i>CLV1</i>	<i>AT1G75820</i>	LRR-RLK	CZ (L2,L3), OC	Promote stem cell differentiation by downregulating WUS. <i>CLV1</i> , and probably <i>CLV2</i> , can bind the <i>CLV3</i> peptide
<i>CLV2</i>	<i>AT1G65380</i>	LRR-RLP	Entire SAM	
<i>CRN</i>	<i>AT5G13290</i>	Kinase	Entire SAM	
<i>RPK2</i>	<i>AT3G02130</i>	LRR-RLK	Periphery, OC	
<i>BAM1</i>	<i>AT5G65700</i>	LRR-RLK	Periphery	Can bind <i>CLE</i> peptides, partially suppress or enhance <i>clv</i> phenotypes
<i>BAM2</i>	<i>AT3G49670</i>	LRR-RLK	Periphery	
<i>BAM3</i>	<i>AT4G20270</i>	LRR-RLK	Periphery	
<i>TDR/PXY</i>	<i>AT5G61480</i>	LRR-RLK	Entire SAM	Could promote stem cell fate
Other factors				
<i>WUS</i>	<i>AT2G17950</i>	Homeodomain transcription factor	OC	Promotes stem cell fate
<i>POL</i>	<i>AT2G46920</i>	Protein phosphatase 2C	Entire SAM	Negative regulators of <i>CLV1</i> , and possibly <i>CRN</i> signaling
<i>KAPP</i>	<i>AT5G19280</i>	Protein phosphatase 2C	Entire SAM	

Genes, ATG numbers, predicted protein types, expression domains, and expected functions of genes related to peptide signaling in the *Arabidopsis* shoot apical meristem.

the formation of larger protein complexes that contain *CLV1*, *CLV2*, and *CRN* (Müller et al. 2008; Bleckmann et al. 2010; also see chapter “Peptides Regulating Root Growth” and “Peptides Regulating Plant Vascular Development”). The genes involved in *Arabidopsis* SAM regulation are listed in Table 1.

4 Feedback Regulation of Stem Cell Fate in Shoot Meristems

The *CLV3* gene was identified as a specific regulator of shoot and floral meristems in 1995. The *clv3* mutants, like *clv1* mutants, have strongly enlarged meristems with a broader stem cell domain and flowers with additional organs. Interestingly, *clv3* mutants are phenotypically stronger than *clv1* mutants, an observation that was later attributed to the second receptor complex, *CLV2/CRN*, still active in *clv1*

mutants (Clark et al. 1995). In 1999, Fletcher et al. identified CLV3 as a small signaling peptide. *CLV3* is specifically expressed in the L1–L3 stem cell layers of the central zone, while *CLV1* is only expressed in deeper layers of the L3. This led to the conclusion that the CLV3 peptide could be secreted from the stem cells where it is produced and bind to the CLV1 or CLV2/CRN receptor complexes in L3 cells (Fletcher et al. 1999). Signaling through the kinase domains of CLV1 or CRN would then lead to the regulation of target genes, thereby restricting stem cell fate.

Since all of the above-mentioned mutants exhibit higher stem cell numbers, the respective proteins are thought to restrict stem cell fate in the wild type. A factor promoting stem cell fate was described in 1996 when it was shown that plants carrying mutations in the *WUSCHEL* (*WUS*) gene terminate growth prematurely and develop flowers that lack the inner organs due to an aberrant differentiation of stem cells (Laux et al. 1996). *WUS* is a homeodomain transcription factor that is expressed in the organizing center (OC), but confers stem cell fate to the cells above in the central zone (Mayer et al. 1998). A later study suggested that *WUS* does not directly influence the expression domain of *CLV3*, but regulates the *CLV3* expression level, probably together with *SHOOTMERISTEMLESS* (*STM*), another homeodomain transcription factor (Brand et al. 2002).

Genetic interaction studies finally connected *CLV* with *WUS* function and allowed establishment of a model for stem cell maintenance in the SAM and floral meristem. Double mutants of *wus* with *clv1* or *clv3* do not maintain an active shoot meristem, thus resembling single *wus* mutants. The flowers of *wus clv1*, *wus clv2*, or *wus clv3* double mutants also resemble *wus* single mutants, indicating epistatic relationship of *wus* toward *clv1*, *clv2*, and *clv3*. *WUS* expression is drastically altered in the *clv* mutants; in all three mutants, the *WUS* expression domain is broader and extends into the lower layer of the central zone. This indicates that the CLV proteins normally function to restrict *WUS* expression from the stem cell domain. Accordingly, *WUS* overexpression causes meristem overgrowth, resulting in plants that resemble *clv* mutants.

Based on these results, it was suggested that the CLV3 peptide is secreted from stem cells and recognized by the CLV receptors in the lower organizing center (OC) cells to restrict *WUS* expression. Reduced *WUS* activity results in fewer stem cells being maintained, thereby lowering CLV3 signal intensity and consequentially strengthening *WUS* expression. Thus, a regulatory feedback loop is established that regulates stem cell number (Schoof et al. 2000). This model is based on the idea that CLV3 is being secreted, a reasonable assumption given the signal peptide in its N-terminal region (also see chapters “Peptides Regulating Plant Vascular Development,” “Processing of Peptides,” and “Methods to Identify New Partners of Plant Signaling Peptides”). The functionality of this signal was tested by Rojo et al. who showed that a full-length version of CLV3 is localized to the extracellular space, while a deletion construct, lacking the signal peptide, remains in the cytoplasm. Accordingly, only the full-length CLV3 version was able to rescue a *clv3* mutant, while the truncated version was not (Rojo et al. 2002). Once secreted, the CLV3 peptide is supposed to diffuse freely in the meristem and is bound by CLV1 and possibly other related RLKs (see below). One immediate consequence of CLV3

binding is a relocation of the CLV1 receptor to the vacuole and receptor degradation. Whether this relocation contributes to the signaling mechanism or serves to attenuate the signaling is currently not known (Nimchuk et al. 2011).

5 Peptides from the CLE Family Control Diverse Developmental Processes

The CLV3 protein, first described in 1999 by Fletcher et al. was predicted to consist of 96 amino acids and carry an N-terminal 18-amino-acid hydrophobic region that may act as a signal peptide, targeting CLV3 into the secretory pathway (Fletcher et al. 1999). The binding of CLV3 to the CLV1 extracellular domain was first shown in vitro, using membrane fractions of plant protoplasts overexpressing *CLV1* (lacking the kinase domain) and with isotope-labeled CLV3 peptide (Ogawa et al. 2008). In 2010, it was shown that this binding of CLV3 actually leads to phosphorylation of the CLV1 kinase (Betsuyaku et al. 2011). More than 400 RLKs are encoded in the *Arabidopsis* genome, and the majority of these represent orphan receptors that have not yet been paired with a ligand. First bioinformatic searches by Cock and McCormick in 2001 uncovered that the EMBRYO SURROUNDING REGION (ESR) proteins from *Zea mays* share a 14-amino-acid conserved region with CLV3. ESR proteins are also secreted peptides, and two *clv3* mutations described previously carry mutations inside the 14-amino-acid conserved region, pointing to a functional role of this domain (Fletcher et al. 1999). The conserved 14 amino acids were later termed the CLE domain (for *CLAVATA3/ESR-related*) or CLE-box that turned out to be conserved among family members in diverse plant species (Cock and McCormick 2001). Based on root growth assays, the CLE peptides were further classified into two classes, named A-type or B-type. For this, *Arabidopsis* seedlings were grown on media, containing one of 22 different *Arabidopsis* CLE peptides. Out of these 22, 18 arrested root growth, while 4, CLE41–44, did not. Based on this, CLE41–44 were classified B-type CLE peptides, and all the others as A-type, even though most subsequent studies focused only on a few peptides from each class (CLE3, CLE6, CLE19, and CLE40 representing the A-type and CLE41 representing the B-type). Concerning the SAM, treatment with A-type CLEs led to stem cell differentiation, while treatment with CLE41 did not, hinting at the possibility that A-type CLEs might regulate primary meristem formation (see chapter “Peptides Regulating Root Growth”), while B-type CLEs control other processes such as vascular differentiation (Whitford et al. 2008; see chapter “Peptides Regulating Plant Vascular Development”). In a follow-up study, overexpression of CLE1, CLE6, and CLE7 was shown to lead to stem cell differentiation in the SAM only, while CLE14 led to the termination of both SAM and root meristem (Meng and Feldman 2010).

CLE42 and tracheary element differentiation inhibitory factor (TDIF) (CLE41/44) have an almost identical amino acid sequence and are believed to be involved in the development of tracheary elements (Ito et al. 2006; see chapters “Peptides

Regulating Plant Vascular Development” and “Methods to Identify New Partners of Plant Signaling Peptides”). A recent study has uncovered that both peptides can promote the initiation of auxiliary meristems. Furthermore, CLE42 but not TDIF is expressed also in the SAM. The receptor for CLE42 is TDIF RECEPTOR (TDR), also known as *PHLOEM INTERCALATED WITH XYLEM* (PXY). The *tdr* mutants are insensitive to CLE42 treatment, and TDR too is expressed in the center of the SAM, overlapping with the CLE42 expression domain. This may indicate that TDR might act as receptor for CLE42 not only in the vascular system but also in the SAM. Furthermore, overexpression of CLE42 and TDIF led to increased and ectopic STM expression, a marker for stem cells. This observation implies that CLE42 signaling also contributes to the establishment and maintenance of shoot meristems (Yaginuma et al. 2011).

6 Posttranslational Processing of CLE Peptides

In 2006, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) from CLV3 overexpressing calli allowed the identification of a 12-amino-acid peptide as the active form of CLV3. This peptide was shown to be hydroxyprolinated on two of its three proline residues, revealing the mature CLV3 peptide sequence to be RTVP^hSGP^hDPLHH. The functionality of this peptide was tested by growing *Arabidopsis* seedlings on growth media containing 1 μ M of the peptide and resulted in severely reduced root growth, reduced SAM size, and phyllotactic defects resembling those of *CLV3* overexpressing lines or *wus* mutants. Synthetic peptides consisting of the 14 amino acid CLE-box, or versions containing the 14-amino-acid conserved region plus up to 14 additional amino acids at the C-terminal end, did lead to the same result; however, an 11 amino acid version seemed inactive. Treatment with 12 amino acid versions of CLE40 and CLE19 led to similar results, showing that these peptides might function in a manner similar to that of CLV3. Proposed functions for the hydroxyproline included peptide stabilization, trafficking, or storage (Kondo et al. 2006). Further analysis of the posttranslational modifications of CLV3 was performed in 2009 by Ohyama et al. who used nano-LC-MS/MS to purify CLE peptides from plant tissue and detected a 13-amino-acid form of CLV3. The authors confirmed the hydroxyl modification of at least one proline residue and detected a sugar chain linked to the proline at position 7. This sugar side chain was found to consist of three L-arabinose residues, making CLV3 a glycopeptide. Functional testing of these peptides and binding affinity to CLV1 showed that it was more active than the other versions of CLV3, in that treatment of *clv3* mutants with this modified glycopeptide led to a full rescue in concentrations in which the other forms could not fully restore the wild type. From this, it was concluded that this version of CLV3 is the candidate closest

to the natural form. As glycosylation is known to affect protein conformation, binding specificity, and stability, it is conceivable that these modifications of CLV3 are essential for biological function and/or protect the mature peptide from active degradation by proteolysis (Ohshima et al. 2009; also see chapters “Peptides Regulating Plant Vascular Development” and “Processing of Peptides”). Meng et al. investigated how CLE peptides obtain their functional specificity and found evidence that regions outside the *CLE*-box are also important for CLE peptide activity (Meng et al. 2010).

7 Receptor Redundancies and Additional CLE-Dependent Pathways

BAM receptors were identified as additional receptor kinases contributing to stem cell specification. When searching for genes with sequence similarity to *CLV1*, DeYoung et al. identified the three *BARELY ANY MERISTEM* (*BAM1-3*) genes. Despite the high similarity between the *BAM* and *CLV1* genes, *bam* mutants show opposite effects to *clv1* mutants in that their meristems are reduced and terminate early and their flowers have less organs. Since *CLV1* genes and *BAM* genes can at least partially replace each other if overexpressed, these differences in function must be caused by different expression patterns. Consistent with this, *BAM* genes are preferentially expressed in the flanks of the meristem, and only faintly in the *CLV1*-expressing domain (DeYoung et al. 2006). Genetic analysis revealed that the *bam1 bam2* mutations enhanced the *clv1* phenotype, but had no effect on the *clv2* phenotype and suppressed the *clv3* phenotype. In a first attempt to explain these effects, a previously described sequestration model was invoked. It was proposed that the BAM receptors protect the central zone from CLE peptides that are expressed in the lateral meristem or young organs by binding and sequestering them. In *bam* mutants, these peptides would be able to reach the center of the meristem, where they could activate *CLV1*, leading to a smaller meristem. These peptides would partially substitute for a lack of CLV3 in *clv3* mutants, and this is consistent with a partial suppression of *clv3* phenotypes by *bam* mutants. The enhancement of the *clv1* phenotype by mutations in *bam1* and *bam2* was explained by a low expression of the BAM receptors in the center of the meristem where they would act redundantly with CLV1 (DeYoung and Clark 2008). This last point was later substantiated when interactions between the BAM and CLV1 receptors were shown in co-immunoprecipitation experiments. Furthermore, it was shown that BAM1 and BAM2 are capable of binding a variety of different CLE peptides using in vitro binding assays, including CLV3 (Guo et al. 2010). It should be noted that the *bam1 bam2 clv1* triple mutants revealed phenotypic traits like strong dwarfism and filamentous floral organs that were not observed in any of the single mutants. This supports the notion that *BAM* and *CLV1* might also act synergistically in other developmental pathways (DeYoung and Clark 2008).

RECEPTOR-LIKE PROTEIN KINASE 2/TOADSTOOL 2 (RPK2/TOAD2) was identified in a genetic screen for suppressors of CLE-induced shoot meristem growth arrest. The *rpk2* mutants resemble weak *clv* mutants and generate an enlarged shoot meristem due to stem cell proliferation and the formation of additional floral organs. Since the effects of *rpk2* are additive to *clv1* or *clv2*, RPK2 probably functions in an independent pathway in parallel to those controlled by CLV1 and CLV2/CRN. This idea is supported by the finding that RPK2 does not interact with CLV1 or CLV2/CRN in transient expression experiments in *Nicotiana benthamiana*, but only forms homomers (Kinoshita et al. 2010). However, co-immunoprecipitation experiments suggested weak interactions between CLV1 and RPK2 (Betsuyaku et al. 2011). Whether RPK2 also binds CLV3 has not yet been determined, but *rpk2* plants seem to be insensitive to the effects of exogenous CLV3 application, such as the reduction of SAM size and root meristem arrest (Kinoshita et al. 2010). Interestingly, RPK2 appears to be depleted from the central zone of the meristem, suggesting that similar to CLV1, the binding of CLV3 to RPK2 may trigger receptor degradation.

8 Downstream Signaling Components

The signaling components acting downstream of the CLV3 perceiving receptor kinases are mostly unknown. Genetic screens have so far identified two different types of phosphatases that are involved in signal attenuation or transduction. In 1997, the kinase-associated protein phosphatase (KAPP) was identified and reported to bind to and dephosphorylate the CLV1 kinase, raising the possibility of KAPP as a negative regulator of CLV signaling. This was supported by the observation that plants overexpressing *KAPP* resemble *clv1* mutants (Williams et al. 1997). However, KAPP is likely involved in a number of different signaling pathways and does not act only upon CLV1. In 2000, the *poltergeist* (*pol*) mutant was identified, and while *pol* single mutants do not have an obvious phenotype, they suppress even strong *clv1*, *clv2*, and *clv3* phenotypes, placing the POL gene function downstream of the CLVs (Yu et al. 2000). Additionally, *pol* enhances the *wus* phenotype. The *pol wus* double mutants show further reduced embryonic organ formation and an overall reduction of floral organ numbers, placing POL in a pathway parallel to the stem cell promoting function of WUS. Triple mutants of *pol wus* and *clv1* resemble *clv1 wus* double mutants, supporting the notion that POL might function in parallel to WUS, but requires CLV1. Interestingly, POL overexpression is sufficient to stimulate stem cell proliferation and maintenance. POL and the related gene POLTERGEIST-LIKE 1 (PLL1) encode membrane-localized protein phosphatases that were shown to be activated by phospholipids (Yu et al. 2003; Song and Clark 2005; Gagne and Clark 2010). The current model suggests that activation of the CLV receptors could antagonize the phospholipid-dependent stimulation of POL and/or PLL1.

The main target gene subject to regulation by the CLV pathway is *WUS*. This transcription factor acts non-cell-autonomously from the organizing center (OC) to promote stem cell fate in the central zone of the SAM. *WUS* acts in concert with the transcriptional corepressor *TOPLESS* (*TPL*) (Kieffer et al. 2006; Long et al. 2006). Direct target genes downregulated by *WUS* are the A-type *ARABIDOPSIS RESPONSE REGULATOR* (*ARR*) genes *ARR5*, 6, 7, and 15, which allow signaling connection through the CLV pathway with cytokinin signaling, since these *ARR* genes are negative regulators of cytokinin signaling. Of these four related genes, at least *ARR7* and 15 were shown to be involved in SAM regulation (Leibfried et al. 2005). Thus, *WUS* ultimately promotes cytokinin signaling in the meristem. The phytohormone auxin again promotes expression of at least *ARR7* and 15, thereby antagonizing the functions of cytokinin and *WUS* (Zhao et al. 2010).

9 Evolutionary Conservation of Peptides and Signaling Pathways Directing Shoot Meristem Maintenance

9.1 CLV-Like Pathways in Dicotyledonous Plants

In 2002, the *Petunia* hybrid *WUS* homolog *TERMINATOR* (*TER*) was described. Just like *wus* mutants in *Arabidopsis*, *ter* mutants in *Petunia* do not maintain their stem cells. In wild type, *TER* is expressed in the central zone underneath the stem cells of the SAM. *TER* acts in parallel with a second transcription factor *HAIRY MERISTEM* (*HAM*) that belongs to the GRAS domain family. In *ham* mutants, the meristems cannot be maintained postembryonically and therefore terminate. Interestingly, *HAM* is only expressed in differentiated cells of organ primordia, sites of organ initiation, and the provascular tissue, but not in the meristem itself. Thus, *HAM* must act non-cell-autonomously. Since mutations in both genes led to meristem termination, the relationship between these transcription factors was analyzed. The *ham ter* double mutants exhibit a hybrid phenotype of traits that lead to the conclusion that they are likely to function in parallel to maintain stem cell activity in the meristem (Stuurman et al. 2002). To date, the identification of the highly similar *Arabidopsis* *WUS* homolog *PhWUS* (*TER*) is the only indication of a possible conservation of the *CLAVATA* pathway also in *Petunia*.

A *WUS* homolog for *Antirrhinum*, *ROSULATA* (*ROA*), was identified in 2006 and the *roa* mutants initiate meristems, but fail to maintain them. Like *WUS* in *Arabidopsis*, *ROA* is expressed below the stem cells (Kieffer et al. 2006). Next to the homeodomain, which is 83% identical between *WUS* and *ROA* and 100% identical between *PhWUS* (*TER*) and *ROA*, sequence analysis revealed three more conserved domains: an acidic domain (Mayer et al. 1998), the *WUS* box, and an ASLELXLN motif (Stuurman et al. 2002). Deletion of these three elements in the *roa-2* mutant resulted in a dominant-negative mutation. Expression of a similarly truncated version of *WUS* in *Arabidopsis* also caused dominant-negative effects,

i.e., loss of meristem maintenance. Since the ASLELXLN motif is similar to known motifs of transcriptional repressors (e.g., the EAR motif), it was proposed that WUS and its homologs function as repressors that recruit transcriptional corepressors via their conserved C-terminal domain. If this repression is relieved by deleting the C-terminal part of WUS or ROA, stem cells are no longer prevented from entering differentiation, and the meristem terminates (Kieffer et al. 2006).

9.2 CLV-Like Pathways in Monocotyledonous Plants

An *in silico* screen for CLV3 homologs by Cock and McCormick (2001) brought up the *embryo-surrounding region* (ESR) genes from *Z. mays*. The sequence homology was confined to the 14 amino acid CLE domain and the functional signal peptide at the N terminus. Since ESR peptides are specifically expressed between the endosperm and embryo, it was suggested that they act as signaling molecules to coordinate development in zygotic and maternal tissue (Opsahl-Ferstad et al. 1997). In *Oryza sativa* (rice), Chu et al. (2006) described FLORAL ORGAN NUMBER 4 (FON4) as an ortholog of the *Arabidopsis* CLV3 peptide. The *fon4* mutants have enlarged shoot and floral meristems that produce more organs and, in the case of the floral meristem, do not terminate after the initiation of the carpel primordia. FON4, like CLV3, contains a CLE motif and a signal peptide for secretion. *FON4* is expressed only in the central apex of the meristem. The functional conservation of the CLV3 CLE motif was further tested by treating *Arabidopsis* and rice seedlings with CLV3 or FON4 peptide. CLV3 peptide induced the premature differentiation and growth arrest of both SAM and root meristem, while FON4 arrested SAM development, but had no effect in the root (Chu et al. 2006).

Homologs of CLV1 or CLV2 were found in *Z. mays* in 2001 and 2005, respectively. *FASCIATED EAR 2* (*FEA2*) was identified in a mutant screen, because *fea2* mutants exhibit larger inflorescences and floral meristems. *FEA2* encodes a LRR receptor-like protein with high sequence similarity to AtCLV2 that localizes to the plasma membrane (Taguchi-Shiobara et al. 2001). The THICK TASSEL DWARF1 (*TD1*) gene of maize was shown to restrict meristem size, and the *td1* mutants show enlarged inflorescence and floral meristems. *TD1* encodes a LRR RLK that is expressed more broadly in the plant compared to *CLV1* of *Arabidopsis*. Because *td1 fea2* double mutants show additive effects, it is possible that both CLV1 and CLV2 orthologs act in parallel pathways in meristem regulation, just like they do in *Arabidopsis* (Bommert et al. 2005). The CLV1 ortholog in *O. sativa* is *FLORAL ORGAN NUMBER 1* (*FON1*). While *CLV1* is expressed specifically in the central region of the meristem, *FON1* is expressed throughout the SAM and the floral meristem, which could indicate that regional specificity of FON1 function depends on a localized cofactor. So far, a CLV2 homolog has not been identified in rice (Suzaki et al. 2009).

WUS homologs were identified in *Z. mays* (*ZmWUS1* and *ZmWUS2*) and in *O. sativa* (*OsWUS*). In contrast to *Arabidopsis* WUS, the maize and rice WUS genes

are expressed in the meristems in a pattern overlapping with that of the *CLV1* homolog *TD1*. *OsWUS* is expressed in leaf primordia and transiently in the SAM. *ZmWUS1* is expressed in the SAM, while *ZmWUS2* is expressed only in young leaves. Thus, *OsWUS* combines the expression patterns of both *ZmWUS* paralogs, which indicates that the two maize paralogs probably evolved from a single gene through duplication (Nardmann and Werr 2006).

10 Conclusion

The cells in the SAM are ultimately responsible for the generation of the majority of the plant body. Their growth and division rates and the identity and differentiation of daughter cells generated at the shoot meristem must be integrated and adapted to the requirements of the plant. Growth patterns will vary with changing temperatures, and nutrient or water availability, but also upon induction of flower formation. Thus, the meristem itself, but also the separate functional regions within a meristem, will need to perceive and respond to both extrinsic and intrinsic signals. Across the meristem, the responses of cell groups to these signals need to be integrated. Peptide signals have so far been shown to coordinate the development of the organizing center with the stem cell zone at the meristem tip. The responsible signaling pathways have been only partially elucidated, and we are just beginning to unravel the cross talk with hormonal signaling pathways. For example, CLV3 signaling from the stem cells can confine WUS expression, and thereby repress cytokinin signaling. While the generation of the CLV3 signal is strictly dependent on stem cells, cytokinin is both generated and perceived by many plant cells and feeds back upon CLV signaling by regulating the expression of the CLV receptors. We expect that other growth-regulating hormones, the prime candidate being auxin, will also modify the CLV communication pathway. How stem cell behavior in the shoot meristem is adjusted to plant growth requirements is not yet understood. Another layer of complexity in peptide signaling can be expected since a range of different peptides is generated in the shoot meristem. These peptides can be proteolytically processed and further modified by glycosylation, thus generating a large array of different signaling molecules. Furthermore, several hundred RLKs are expressed in meristems. Those that were already analyzed revealed the capacity for complex formation of hetero- or homomers, suggesting that even cells expressing only a limited number of receptors can form a dazzling number of different potential receptor complexes. We can expect that meristem cells employ a large number of peptide-controlled signaling pathways for communication between meristem domains. Redundant or parallel acting pathways are likely to complicate their genetic dissection, and we will need to improve our biochemical toolbox to unravel how specific peptides regulate diverse receptor complexes in meristem domains and how they control plant growth and differentiation.

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