Ready, Aim, Shoot: Stem Cell Regulation of the Shoot Apical Meristem

Cara L Soyars¹, Sean R James¹, and Zachary L Nimchuk¹

¹ Department of Biology, University of North Carolina at Chapel Hill, 4155
Genome Sciences Building, 250 Bell Tower Drive, Chapel Hill, NC, 27599

Corresponding author: Nimchuk, Zachary L (zackn@email.unc.edu)

Abstract

Plant shoot meristems contain stem cells that are continuously renewed to replenish cells that exit and differentiate during lateral organ formation. Complex cell-to-cell signaling systems balance division and differentiation. These center on ligand-receptor networks, hormone pathways, and transcriptional regulators that function in an integrated manner. In this review, we aim to highlight new findings in shoot stem cell regulation across species.

Introduction

The logic of plant development necessitates both continuous renewal and plasticity in form. In above ground tissues, this task falls on the shoot apical meristem (SAM), which acts as a reservoir of pluripotent stem cells that is necessary for the development of leaves, floral meristems (FMs), and shoots throughout the life of the plant. The SAM is organized in three distinct cell layers (L1-L3) (Figure 1a). L1 stem cells generate epidermal tissue, while L2 and L3 will give rise to ground tissues and vascular tissues respectively. Imposed over this layering, is a zonation determined by gene expression and function, with signals from the central organizing center (OC) regulating stem cell maintenance in the overlaying central zone (CZ) (Figure 1b). When the stem cells divide, some of their progeny are laterally displaced into the peripheral zone (PZ), where they will proliferate and ultimately differentiate during organogenesis. Cells that are basally displaced to the rib meristem (RM) contribute to internal shoot tissues.

The spatiotemporal zonation in the SAM is maintained by cell-to-cell signaling systems that specify cell specific transcriptional activity in the different zones to balance cellular division and regulate differentiation. Decades of research have uncovered conserved key regulators and networks that operate across species in SAM function. However, it is becoming clear that the wiring of these pathways intersects and diverges in significant ways across species. In this review we

highlight new research that furthers our understanding of these pathways, and how they have been tweaked by evolution and domestication.

Cellular communication by receptor kinase-peptide signaling networks

The size of the SAM stem cell pool depends on intercellular communication between the OC and the CZ. The CLAVATA3 peptide (CLV3p) is secreted from CZ cells and diffuses into the underlying OC where it is perceived by an overlapping series of receptor kinases that signal to dampen the expression of the pro-stem cell homeodomain transcription factor WUSCHEL (WUS) [1-3]. This pathway was first defined in Arabidopsis but is conserved in flowering plants such as maize, rice and tomato. Mutations that effect CLV3p production or perception lead to increases in stem cell production in SAMs. WUS moves from the OC to the stem cells in the CZ via plasmodesmata where it promotes stem cell activity [4,5]. WUS limits its own activity by promoting *CLV3* expression, forming a feedback loop to maintain the size of the stem cell niche [1,3,5] (Figure 2).

The mature CLV3p is a 13 amino acid glycopeptide modified with three L-arabinose residues [6-8] In Arabidopsis, this modification promotes high affinity binding to receptors [8]. Arabinosyltransferase enzymes catalyze the attachment and extension of the arabinose chain including HYDROXYPROLINE O-ARABINOSYLTRANSFERASE 3 (HPAT3) [9], REDUCED RESIDUAL ARABINOSE 3 (RRA3) [10], and XYLOGLUCANASE 113 (XEG113) [11]. In tomato, mutations in this pathway strongly reduces CLV3p potency, causing meristem enlargement [12]. *FASCIATED AND BRANCHED (FAB)* encodes the tomato homolog of *CLAVATA1 (CLV1)*, while *FASCIATED INFLORESCENCE (FIN)* encodes a HPAT protein most similar to Arabidopsis HPAT3 shown in both systems to modify CLV3 related peptides [9,12]. Consistent with this, tomato *fin* mutants are rescued by the addition of arabinosylated CLV3p [12]. Interestingly, *hpat* mutations in Arabidopsis have little effect on SAM function. The classical

fasciated (*fas*) mutant of tomato was shown to be a *CLV3* promoter variant that reduces *CLV3* expression [12]. In contrast, *Arabidopsis* tolerates similar reductions in *CLV3*, supporting the idea that the two systems are differentially sensitized to CLV3p [13].

CLV3p is perceived by overlapping leucine-rich repeat receptor kinases (LRR-RKs) composed of CLV1 and the related BARELY ANY MERISTEM 1, 2, and 3 (BAM 1, 2, and 3) RKs [14] and the more distantly related LRR-RK RECEPTOR-LIKE PROTEIN KINASE 2 (RPK2) [15]. CLV3p perception also requires the heterodimer of the non-kinase LRR CLV2 and the pseudokinase CORYNE (CRN) [16-18]. The role of CRN/CLV2 is enigmatic. Genetic and cell biological evidence suggests that CLV2/CRN are dispensable for the perception of CLV3p and subsequent CLV1 signaling [16,19], yet they are unlikely to signal on their own owing to the lack of kinase activity in CRN [20]. Recent work using the tobacco transient system has suggested that CLV2/CRN may function to promote the formation of higher order CLV1 complexes potentially amplifying signaling [21].

New data demonstrate that the genetic redundancy in the CLV1-BAM clade is a consequence of transcriptional cross regulation. *CLV1* in the RM is necessary and sufficient for wild-type function [22]. In wild-type SAMs, *CLV1* actively signals via CLV3p to repress *BAM* expression from the RM. In *clv1* mutant SAMs, *BAM1* and *BAM3* are ectopically expressed in the RM, partially compensating for *clv1* loss of function [22]. Consistently, both BAM1 and BAM2 can also bind CLV3p [23]. Quadruple null mutants (*bam1 bam2 bam3 clv1*) exhibit SAM over-proliferation that is stronger than *clv3* null mutants [22], pointing to the existence of redundant ligands. Ligand redundancy in SAM regulation is a common theme shared in rice [24] and tomato [12].

The ERECTA (ER) LRR-RK pathway operates in parallel with CLV1 to regulate the expression of *WUS* [25]. The ligands for ER here are unknown, but are likely

to be members of the EPIDERMAL PATTERNING FACTOR (EPF) family that are involved with plant stature and stomatal development [26]. Although they are distinct clades, CLV1 can substitute for ER in some cases [27]. ER signals via a MAPK signaling cascade in stomatal development [28] and there is some evidence that MAPK signaling may function downstream of CLV1 [29]. ER has also been proposed to signal via heterotrimeric G proteins [30]. The maize G α subunit is required for CLV3p perception and directly interacts with the CLV2 ortholog FASCIATED EAR2 (FEA2) [31]. In a twist, Arabidopsis mutations in G *protein beta-subunit1* (*agb1*), but not G α , result in an enlarged SAM phenotype similar to *clv* mutants [32]. Genetic and biochemical analysis suggests that AGB1 and RPK2 interact [32]. These results establish the involvement of G-proteins as signaling molecules in meristem development and suggest that ER and CLV class receptors may converge on a common signaling path. Signaling via the CLV pathway is negatively regulated by a series of protein phosphatases including POLTERGIEST, POL-LIKE1 and Kinase Associated Protein Phosphatase (KAPP) [33,34], but the direct connection to receptor function remains unclear. Recent work has also indicated that an E3 ubiquitin ligase, PLANT U-BOX (PUB4), may function in CLV3p signaling [35].

Transcriptional outputs of peptide receptor pathways

In the RM, WUS acts as both a repressor and activator to regulate genes involved in stem cell differentiation and maintenance of the stem cell niche [2,36], where it buffers SAM function under stress [36]. WUS impacts the transcription of not just CLV3, but a suite of genes involved in cell proliferation, cell differentiation, and cytokinin signaling [37,38]. WUS is partially interchangeable with related WUSCHEL-related homeobox (WOX) family members [39]. Zhou et al. has demonstrated that the HAIRY MERISTEM (HAM) GRAS domain transcription factors partner with WUS/WOX to cooperatively control the shoot stem cell niche through regulation of a common gene set [40]. *ham1;2;3;4* quadruple homozygous mutants phenocopy mutants that lack WOX function [40].

This pathway is conserved in *Petunia* [41] and promotes SAM maturation in rice [42].

Transcriptional integration with hormone signaling systems

As cells leave the CZ and enter the PZ, they begin a path towards lateral organ formation and differentiation. This process is governed by local accumulation of the hormone auxin on the PZ flanks driven by polar localization of the PIN-FORMED1 (PIN1) auxin efflux transporter, subsequent transcriptional regulation by the auxin response factor MONOPTEROS (MP), and additional transcriptional regulators, which act in part to amplify the auxin response in developing primordia [43]. Auxin transported from the leaves to the SAM creates a low auxin zone in the leaves, which promotes correct adaxial-abaxial patterning of the leaves [44]. In the monocot *Brachypodium*, PIN1 is involved in primordia specification and associated vascularization and employ two PIN genes as a result of a conserved duplication of *PIN1* in grasses [45]. Auxin also impacts lateral organ formation in part through regulating cell wall remodeling and cell expansion, and altering mechanical properties of the SAM. By interfering with cortical microtubule anisotropy, auxin allows for destabilization of the vertical growth axis and generates axes of growth for lateral organs [46]. The positioning of lateral organs is intimately tied to SAM size, and mutations that expand SAM size alter phyllotaxy [47,48].

Cytokinin (CK) also plays a role in controlling SAM architecture and activity by inhibiting differentiation of stem cells in the SAM and promoting cell division. CK promotes SAM enlargement in part through *WUS*, and CK synthesis or perception defects reduce SAM size [49,50]. REPRESSOR OF CYTOKININ DEFICIENCY 1 (ROCK1), an ER-localized nucleotide-sugar transporter, functions in this pathway to regulate the stability of CK-degrading enzymes and control SAM function [51]. ROCK1 fine tunes CK activity through modification of

CK degrading proteins [51]. Mutations in *ALTERED MERISTEM PROGRAM1* (AMP1) generate ectopic stem cell niches and have increased leaf formation. These effects were previously contributed to increased CK levels present in the mutant, but Huang et al. has shown that *amp1* SAM phenotypes are largely CK independent [52]. AMP1 regulates mircoRNA function suggesting that mircoRNA targets may underlie *amp1* phenotypes [53].

WUS cooperates with the SAM promoting transcription factor SHOOT MERISTEMLESS (STM) to modulate hormone status in the SAM. STM stimulates CK biosynthesis in the SAM by promoting *ISOPENTYLTRANSFERASE7* (*IPT7*) expression and WUS acts to create a cytokinin maxim in the OC via repression of *ARABIDOPSIS RESPONSE REGULATOR 7* (*ARR7*) and *ARR15* [49]. WUS also represses the expression of *HECATE* family transcriptional regulators from the OC, which also regulate CK responsiveness and *CLV3* levels [38]. In addition, *STM* plays additional roles in preventing giberrellin (GA) accumulation, maintaining *WUS* expression, and preventing SAM cells from adopting lateral organ-specific identities [54]. Studies of diverse plant lineages have indicated that the function of the *KNOX* genes diverged during the evolution of diploid body plans in plants [54]. STM and cytokinin repress *ASSYMETRIC LEAVES1* (*AS1*), a MYB-domain transcriptional repressor of class-1 *KNOX* genes [55]. However, a new model suggests that *STM* and *AS1* competitively control *KNOX* gene expression [56]. In addition, Tsuda et al. has determined that OSH1, a homologue of STM in rice represses the brassinosteroid (BR) pathway by transcriptionally activating BR catabolic genes [57]. The expression of *WUS* is also buffered by class III Homeodomain leucine zipper transcription factors, which themselves have been implicated in shaping hormone networks outside of the SAM [58].

While the contributions of WUS and STM to SAM control in flowering plants are highly conserved, the functions of other regulators have diverged. *FASCIATED EAR4* (*FEA4*) has been identified as a basic region/leucine zipper transcription

factor that is orthologous to Arabidopsis *PERIANTHIA* (PAN) which promotes the homeotic MADS domain *AGAMOUS* (AG) to repress *WUS* during stem cell termination in FMs [59,60]. Pautler et al. has demonstrated that FEA4 binds a maize ortholog of AG in the third intron as compared to the Arabidopsis ortholog PAN which binds the AG second intron [59]. While FEA4 and PAN have comparable functions in flower formation, FEA4 also plays a critical role in inflorescence and vegetative meristem size, something not seen in Arabidopsis despite PAN being expressed in the SAM. It has also been suggested that FEA4 is a target of the glutaredoxin MALE STERILE CONVERTED ANTH1 (MSCA1) [61]. Dominant mutants in *MSCA1* exhibit an enlarged SAM and decussate phyllotaxy, while loss of function mutations have a reduction in SAM size and wild-type alternate phyllotaxy [61].

Conclusions and future directions

The regulation of spatiotemporal zones within the dynamic tissue of the SAM involves the communication and coordination of transcriptional networks, hormone signaling pathways, and transcriptional regulator trafficking (Table 1). Ultimately these impact cell cycle progression and differentiation. Recent work has begun to unravel the interlocking networks. Future research aimed at connecting hormone and receptor pathways to downstream transcriptional regulators will be critical for defining specific signaling pathways. Understanding how these networks modulate cell cycle and morphology is a future challenge. Recent automated imaging and modeling systems will likely aid in this process.

Acknowledgements

Work in the Nimchuk lab is supported by startup funds provided from The University of North Carolina at Chapel Hill, and from the NSF IOS program (IOS-1455607). We would like to extend our apologies to colleagues whose work could not be included in this review due to length constraints.

References and Recommended Reading

1. Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R: **Dependence of stem cell fate in Arabidopsis on a feedback loop regulated by CLV3 activity.** *Science* 2000, **289**:617-619.
2. Mayer KFX, Schoof H, Haecker A, Lenhard M, Jürgens G, Laux T: **Role of WUSCHEL in Regulating Stem Cell Fate in the Arabidopsis Shoot Meristem.** *Cell* 1998, **95**:805-815,0092-8674.
3. Schoof H, Lenhard M, Haecker A, Mayer KFX, Jürgens G, Laux T: **The Stem Cell Population of Arabidopsis Shoot Meristems Is Maintained by a Regulatory Loop between the CLAVATA and WUSCHEL Genes.** *Cell* 2000, **100**:635-644, 0092-8674.
4. Daum G, Medzhradszky A, Suzuki T, Lohmann JU: **A mechanistic framework for noncell autonomous stem cell induction in Arabidopsis.** *Proceedings of the National Academy of Sciences* 2014, **111**:14619-14624.
5. Yadav RK, Perales M, Gruel J, Girke T, Jönsson H, Reddy GV: **WUSCHEL protein movement mediates stem cell homeostasis in the Arabidopsis shoot apex.** *Genes & development* 2011, **25**:2025-2030.
6. Ohyama K, Shinohara H, Ogawa-Ohnishi M, Matsubayashi Y: **A glycopeptide regulating stem cell fate in Arabidopsis thaliana.** *Nature chemical biology* 2009, **5**:578-580, 1552-4450.
7. Kondo T, Sawa S, Kinoshita A, Mizuno S, Kakimoto T, Fukuda H, Sakagami Y: **A plant peptide encoded by CLV3 identified by in situ MALDI-TOF MS analysis.** *Science* 2006, **313**:845-848.
8. Shinohara H, Matsubayashi Y: **Chemical synthesis of Arabidopsis CLV3 glycopeptide reveals the impact of hydroxyproline arabinosylation on peptide conformation and activity.** *Plant and Cell Physiology* 2013, **54**:369-374.
9. Ogawa-Ohnishi M, Matsushita W, Matsubayashi Y: **Identification of three hydroxyproline O-arabinosyltransferases in Arabidopsis thaliana.** *Nature chemical biology* 2013, **9**:726-730.
10. Egelund J, Obel N, Ulvskov P, Geshi N, Pauly M, Bacic A, Petersen BL: **Molecular characterization of two Arabidopsis thaliana glycosyltransferase mutants, rra1 and rra2, which have a reduced residual arabinose content in a polymer tightly associated with the cellulosic wall residue.** *Plant molecular biology* 2007, **64**:439-451.
11. Gille S, Hänsel U, Ziemann M, Pauly M: **Identification of plant cell wall mutants by means of a forward chemical genetic approach using hydrolases.** *Proceedings of the National Academy of Sciences* 2009, **106**:14699-14704.
- **12. Xu C, Liberatore KL, MacAlister CA, Huang Z, Chu Y-H, Jiang K, Brooks C, Ogawa-Ohnishi M, Xiong G, Pauly M: **A cascade of arabinosyltransferases controls shoot meristem size in tomato.** *Nature genetics* 2015.

- This study demonstrated the role of arabinosyltransferase genes as critical players in the CLV pathway. They found that the mature CLV3 peptide must be fully arabinosylated in order to regulate meristem size and that CLV3 mutations resulted in an increased fruit size during the process of tomato domestication. Their findings suggest that arabinosyltransferase family members have been coopted to regulate signaling peptides across a variety of species and developmental contexts.
13. Müller R, Borghi L, Kwiatkowska D, Laufs P, Simon R: **Dynamic and compensatory responses of Arabidopsis shoot and floral meristems to CLV3 signaling.** *The Plant Cell* 2006, **18**:1188-1198.
 14. DeYoung BJ, Bickle KL, Schrage KJ, Muskett P, Patel K, Clark SE: **The CLAVATA1- related BAM1, BAM2 and BAM3 receptor kinase- like proteins are required for meristem function in Arabidopsis.** *The Plant Journal* 2006, **45**:1-16.
 15. Kinoshita A, Betsuyaku S, Osakabe Y, Mizuno S, Nagawa S, Stahl Y, Simon R, Yamaguchi-Shinozaki K, Fukuda H, Sawa S: **RPK2 is an essential receptor-like kinase that transmits the CLV3 signal in Arabidopsis.** *Development* 2010, **137**:3911-3920.
 16. Müller R, Bleckmann A, Simon R: **The receptor kinase CORYNE of Arabidopsis transmits the stem cell-limiting signal CLAVATA3 independently of CLAVATA1.** *The Plant Cell* 2008, **20**:934-946.
 17. Jeong S, Trotochaud AE, Clark SE: **The Arabidopsis CLAVATA2 gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase.** *The Plant Cell* 1999, **11**:1925-1933.
 18. Bleckmann A, Weidtkamp-Peters S, Seidel CA, Simon R: **Stem cell signaling in Arabidopsis requires CRN to localize CLV2 to the plasma membrane.** *Plant physiology* 2010, **152**:166-176.
 19. Nimchuk ZL, Tarr PT, Ohno C, Qu X, Meyerowitz EM: **Plant stem cell signaling involves ligand-dependent trafficking of the CLAVATA1 receptor kinase.** *Current Biology* 2011, **21**:345-352.
 20. Nimchuk ZL, Tarr PT, Meyerowitz EM: **An evolutionarily conserved pseudokinase mediates stem cell production in plants.** *The Plant Cell* 2011, **23**:851-854.
 - *21. Somssich M, Ma Q, Weidtkamp-Peters S, Stahl Y, Felekyan S, Bleckmann A, Seidel CA, Simon R: **Real-time dynamics of peptide ligand-dependent receptor complex formation in planta.** *Sci. Signal.* 2015, **8**.
By using a multiparameter fluorescence imaging spectroscopy (MFIS) approach, this study measured changes in protein concentration and homomeric and heteromeric interactions between receptors of the CLV and FLS2 signalling pathways. They showed that the CLV receptors were in pre-formed complexes before the addition CLV3p which suggests that these receptors are in a “ready” state for fast detection of CLV3p. In contrast, BAK1/FLS2 receptor complexes formed only after the addition of the ligand.

- **22. Nimchuk ZL, Zhou Y, Tarr PT, Peterson BA, Meyerowitz EM: Plant stem cell maintenance by transcriptional cross-regulation of related receptor kinases.** *Development* 2015, **142**:1043-1049.
Nimchuk and colleagues have provided an explanation for the genetic redundancy observed in the CLV1 clade of RKs in which the *BAM* genes are repressed by CLV1 signaling. It was shown that in wild-type SAMs *CLV1* is expressed in the RM, and actively signals via CLV3p to repress *BAM* expression from the RM. However, in *clv1* mutant SAMs, *BAM 1* and *BAM3* are ectopically expressed in the RM partially compensating for loss of *CLV1*.
- *23. Shinohara H, Matsubayashi Y: 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.** *The Plant Journal* 2015, **82**:328-336.
In this study, the authors used photoaffinity labeling of the CLV2, RPK2, and BAM receptors to gain evidence of direct ligand-receptor interactions. They also developed a method in which the arabinosylated CLV3 peptide can be chemically synthesized thus allowing for the detection of interactions between CLV3 and receptors via photoaffinity labeling. This study offers new methods to detect ligand-receptor interactions in native signaling pathways.
- 24. Suzuki T, Ohneda M, Toriba T, Yoshida A, Hirano H-Y: FON2 SPARE1 redundantly regulates floral meristem maintenance with FLORAL ORGAN NUMBER2 in rice.** *PLoS Genet* 2009, **5**:e1000693.
- 25. Shpak ED: Diverse roles of ERECTA family genes in plant development.** *Journal of integrative plant biology* 2013, **55**:1238-1250.
- 26. Torii KU: Mix-and-match: ligand-receptor pairs in stomatal development and beyond.** *Trends in plant science* 2012, **17**:711-719.
- 27. Diévert A, Dalal M, Tax FE, Lacey AD, Huttly A, Li J, Clark SE: CLAVATA1 dominant-negative alleles reveal functional overlap between multiple receptor kinases that regulate meristem and organ development.** *The Plant Cell* 2003, **15**:1198-1211.
- 28. Bergmann DC, Lukowitz W, Somerville CR: Stomatal development and pattern controlled by a MAPKK kinase.** *Science* 2004, **304**:1494-1497.
- 29. Betsuyaku S, Takahashi F, Kinoshita A, Miwa H, Shinozaki K, Fukuda H, Sawa S: Mitogen-activated protein kinase regulated by the CLAVATA receptors contributes to shoot apical meristem homeostasis.** *Plant and cell physiology* 2011, **52**:14-29.
- 30. Llorente F, Alonso-Blanco C, Sánchez-Rodríguez C, Jorda L, Molina A: ERECTA receptor-like kinase and heterotrimeric G protein from Arabidopsis are required for resistance to the necrotrophic fungus plectosphaerella Cucumerina.** *The Plant Journal* 2005, **43**:165-180.
- 31. Bommert P, Je BI, Goldshmidt A, Jackson D: The maize G agr gene COMPACT PLANT2 functions in CLAVATA signalling to control shoot meristem size.** *Nature* 2013, **502**:555-558.

32. Ishida T, Tabata R, Yamada M, Aida M, Mitsumasu K, Fujiwara M, Yamaguchi K, Shigenobu S, Higuchi M, Tsuji H: **Heterotrimeric G proteins control stem cell proliferation through CLAVATA signaling in Arabidopsis.** *EMBO reports* 2014:e201438660.
33. Williams RW, Wilson JM, Meyerowitz EM: **A possible role for kinase-associated protein phosphatase in the Arabidopsis CLAVATA1 signaling pathway.** *Proceedings of the National Academy of Sciences* 1997, **94**:10467-10472.
34. Song S-K, Lee MM, Clark SE: **POL and PLL1 phosphatases are CLAVATA1 signaling intermediates required for Arabidopsis shoot and floral stem cells.** *Development* 2006, **133**:4691-4698.
35. Kinoshita A, Seo M, Kamiya Y, Sawa S: **Mystery in genetics: PUB4 gives a clue to the complex mechanism of CLV signaling pathway in the shoot apical meristem.** *Plant signaling & behavior* 2015.
36. Ikeda M, Mitsuda N, Ohme-Takagi M: **Arabidopsis WUSCHEL is a bifunctional transcription factor that acts as a repressor in stem cell regulation and as an activator in floral patterning.** *The Plant Cell* 2009, **21**:3493-3505.
37. Yadav RK, Perales M, Gruel J, Ohno C, Heisler M, Girke T, Jönsson H, Reddy GV: **Plant stem cell maintenance involves direct transcriptional repression of differentiation program.** *Molecular systems biology* 2013, **9**:654.
38. Schuster C, Gaillochet C, Medzihradszky A, Busch W, Daum G, Krebs M, Kehle A, Lohmann JU: **A regulatory framework for shoot stem cell control integrating metabolic, transcriptional, and phytohormone signals.** *Developmental cell* 2014, **28**:438-449.
39. van der Graaff E, Laux T, Rensing SA: **The WUS homeobox-containing (WOX) protein family.** *Genome Biol* 2009, **10**:248.
- **40. Zhou Y, Liu X, Engstrom EM, Nimchuk ZL, Pruneda-Paz JL, Tarr PT, Yan A, Kay SA, Meyerowitz EM: **Control of plant stem cell function by conserved interacting transcriptional regulators.** *Nature* 2015, **517**:377-380.
 With multiple approaches, Zhou and colleagues demonstrated the Arabidopsis family of HAM transcription factors acts as conserved cofactors with WUS/WOX proteins to control the shoot stem cell niche through regulation of a common gene set. A quadruple *ham1;2;3;4* mutant was generated to determine the importance of the entire family in the control the stem cell niche, and resulting mutants had mutations similar to that of mutants that lack WOX function.
41. Stuurman J, Jäggi F, Kuhlemeier C: **Shoot meristem maintenance is controlled by a GRAS-gene mediated signal from differentiating cells.** *Genes & development* 2002, **16**:2213-2218.
42. Fan T, Li X, Yang W, Xia K, Ouyang J, Zhang M: **Rice osa-miR171c Mediates Phase Change from Vegetative to Reproductive Development and Shoot Apical Meristem Maintenance by Repressing Four OsHAM Transcription Factors.** *PloS one* 2015, **10**:e0125833.

43. Yruela I: **PLANT DEVELOPMENT REGULATION: OVERVIEW AND PERSPECTIVES**. *Journal of Plant Physiology* 2015.
44. Qi J, Wang Y, Yu T, Cunha A, Wu B, Vernoux T, Meyerowitz E, Jiao Y: **Auxin depletion from leaf primordia contributes to organ patterning**. *Proceedings of the National Academy of Sciences* 2014, **111**:18769-18774.
- **45. O'Connor DL, Runions A, Sluis A, Bragg J, Vogel JP, Prusinkiewicz P, Hake S: **A division in PIN-mediated auxin patterning during organ initiation in grasses**. *PLoS computational biology* 2014, **10**.
The authors utilized a phylogenetic and gene synteny analysis to identify a clade sister to *PIN1* termed *Sister-of-PIN1*(*SoPIN1*) as well as a conserved duplication of *PIN1* in two grass species. Their results suggested that *SoPIN1* has a role in auxin maximum formation and the conserved duplications are involved in vein patterning. This study provides evidence that the Brassicaceae family has lost a clade of *PIN* genes conserved in all other angiosperms.
46. Sassi M, Ali O, Boudon F, Cloarec G, Abad U, Cellier C, Chen X, Gilles B, Milani P, Friml J: **An auxin-mediated shift toward growth isotropy promotes organ formation at the shoot meristem in Arabidopsis**. *Current Biology* 2014, **24**:2335-2342.
47. Landrein B, Refahi Y, Besnard F, Hervieux N, Mirabet V, Boudaoud A, Vernoux T, Hamant O: **Meristem size contributes to the robustness of phyllotaxis in Arabidopsis**. *Journal of experimental botany* 2014:eru482.
48. Mandel T, Moreau F, Kutsher Y, Fletcher JC, Carles CC, Williams LE: **The ERECTA receptor kinase regulates Arabidopsis shoot apical meristem size, phyllotaxy and floral meristem identity**. *Development* 2014, **141**:830-841.
49. Kozuka J: **Control of shoot and root meristem function by cytokinin**. *Current opinion in plant biology* 2007, **10**:442-446.
50. Leibfried A, To JP, Busch W, Stehling S, Kehle A, Demar M, Kieber JJ, Lohmann JU: **WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators**. *Nature* 2005, **438**:1172-1175.
51. Niemann MC, Bartrina I, Ashikov A, Weber H, Novák O, Spíchal L, Strnad M, Strasser R, Bakker H, Schmölling T: **Arabidopsis ROCK1 transports UDP-GlcNAc/UDP-GalNAc and regulates ER protein quality control and cytokinin activity**. *Proceedings of the National Academy of Sciences* 2015, **112**:291-296.
52. Huang W, Pitorre D, Poretska O, Marizzi C, Winter N, Poppenberger B, Sieberer T: **ALTERED MERISTEM PROGRAM1 suppresses ectopic stem cell niche formation in the shoot apical meristem in a largely cytokinin-independent manner**. *Plant physiology* 2015, **167**:1471-1486.
53. Li S, Liu L, Zhuang X, Yu Y, Liu X, Cui X, Ji L, Pan Z, Cao X, Mo B: **MicroRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in Arabidopsis**. *Cell* 2013, **153**:562-574.

54. Tsuda K, Hake S: **Diverse functions of KNOX transcription factors in the diploid body plan of plants.** *Current opinion in plant biology* 2015, **27**:91-96.
55. Byrne ME, Simorowski J, Martienssen RA: **ASYMMETRIC LEAVES1 reveals knox gene redundancy in Arabidopsis.** *Development* 2002, **129**:1957-1965.
56. Scofield S, Dewitte W, Murray JA: **STM sustains stem cell function in the Arabidopsis shoot apical meristem and controls KNOX gene expression independently of the transcriptional repressor AS1.** *Plant signaling & behavior* 2014, **9**:e28934.
57. Tsuda K, Kurata N, Ohyanagi H, Hake S: **Genome-wide study of KNOX regulatory network reveals brassinosteroid catabolic genes important for shoot meristem function in rice.** *The Plant Cell* 2014, **26**:3488-3500.
58. Turchi L, Baima S, Morelli G, Ruberti I: **Interplay of HD-Zip II and III transcription factors in auxin-regulated plant development.** *Journal of experimental botany* 2015.
- *59. Pautler M, Eveland AL, LaRue T, Yang F, Weeks R, Je BI, Meeley R, Komatsu M, Vollbrecht E, Sakai H: **FASCIATED EAR4 encodes a bZIP transcription factor that regulates shoot meristem size in maize.** *The Plant Cell* 2015, **27**:104-120.
 The authors characterized the bZIP transcription factor *FEA4*, which is the maize ortholog to the Arabidopsis *PAN*. Through genetic analysis, they showed that *FEA4* acts parallel to the CLV pathway. They also determined that *FEA4* is necessary to regulate auxin responses and leaf differentiation in the periphery of the meristem. This study demonstrated the importance of studying basic processes in various models because in order to reveal novel functions.
60. Das P, Ito T, Wellmer F, Vernoux T, Dedieu A, Traas J, Meyerowitz EM: **Floral stem cell termination involves the direct regulation of AGAMOUS by PERIANTHIA.** *Development* 2009, **136**:1605-1611.
- *61. Yang F, Bui HT, Pautler M, Llaca V, Johnston R, Lee B-h, Kolbe A, Sakai H, Jackson D: **A maize glutaredoxin gene, Abphyl2, regulates shoot meristem size and phyllotaxy.** *The Plant Cell* 2015, **27**:121-131.
 Yang and colleagues characterized *Abph2*, which encodes a glutaredoxin gene. The dominant maize mutant *abph2* has an enlarged meristem and a switch to decussate phyllotaxy. Map-based cloning indicated that the *Abph2* mutation was due to transposition of the GRX *MSCA1*. The authors have identified a novel function of GRX genes in meristem growth as well as a novel pathway regulating meristematic size and phyllotaxy involving *MSC1* and *FEA4*.

Figure Captions

Figure 1: Arabidopsis SAM. **(a)** Schematic view of cellular layers in Arabidopsis SAM. The SAM contains three distinct cellular layers (L1-L3). Cells within the L1 (yellow) layer and the L2 (green) layer undergo anticlinal division, while cells within the L3 (turquoise) layer undergo both anticlinal and periclinal division. **(b)** Outline of developmental zones within Arabidopsis SAM. The SAM contains three developmental zones determined by gene expression and function: the central zone (CZ, yellow), rib meristem (RM, green), and the peripheral zone (PZ, purple). Signals from the central organizing center (OC, pink) regulate stem cell maintenance in the overlaying CZ. When the stem cells divide, some of the progeny is displaced to the PZ where they will differentiate and give rise to organ primordia (OP, red). Cells displaced from the RM will contribute to internal shoot tissue.

Figure 2: Signaling networks that regulate stem cell development. CLV3 is produced and modified by HPAT3, RRA3, and XEG113 to make the mature CLV3 peptide (CLV3p). CLV3p is secreted and is perceived by the CLV1 complex and possibly CLV2/CRN and RPK2 complexes. POL and PLL1, two redundant protein phosphatases, negatively regulate the CLV1 signaling pathway. Signaling through the G protein pathway inhibits WUS, HAM, and other genes, which in turn inhibits stem cell division and allows for differentiation of stem cells to occur. See text for more details.

Table 1: Summary of key players involved in stem cell regulation discussed in the review. See text for further details.

Figure 1

Figure 2

Key Player	Molecular Function	Function in Stem Cell Maintenance
CLV3 ¹	Peptide ligand	Binds CLV1 RK to negatively regulate shoot stem cell proliferation
CLV1 ¹⁴	Receptor kinase	CLV3 receptor
WUS ²⁻⁵	Homeodomain transcription factor	Promotes stem cell maintenance and CLV3 expression
HPAT3/FIN ^{9,12} RRA3 ¹⁰ XEG113 ¹¹	Arabinosyltransferases	Modifies CLV3 peptide
BAM 1,2,3 ^{14,22}	Receptor kinase	Negatively regulate stem cell proliferation in absence of CLV1 signaling
CLV2 ¹⁷	Receptor-like protein	Promotes CLV signaling independent of CLV1
CRN ^{16,18,20}	Pseudokinase	Promotes CLV2 plasma membrane localization
RPK2 ¹⁵	Receptor-like kinase	Transmits CLV3 signal independently of CLV1 and CLV2
ER ²⁵⁻²⁹	Receptor-like kinase	Operates in parallel with CLV1 to regulate WUS expression
Gα ³¹	G protein subunit	Interacts with FEA2
AGB1 ³²	G protein subunit	Interacts with RPK2
POL ^{33,34} PLL1	Protein phosphatases	Negatively regulates CLV signaling
HAM 1,2,3,4 ⁴⁰	GRAS domain transcription factors	Partner with WUS/WOX to control stem cell maintenance
PUB4 ³⁵	E3 ubiquitin ligase	Promotes CLV signaling independently of CLV1
PIN1 ⁴⁵	Auxin efflux transporter	Promotes accumulation of auxin
MP ⁴³	Auxin response factor	Mediates auxin response in developing primordia
AMP1 ^{52,53}	Putative carboxypeptidase	Suppresses ectopic stem cell niche formation in SAM, regulates miRNA function
STM ^{49,54}	Class 1 KNOX protein	Stimulates CK biosynthesis, maintains WUS expression, prevents stem cells from adopting lateral organ specific identities
AS1 ⁵⁵	MYB-domain transcriptional repressor	Competitively controls KNOX gene expression with STM
PAN/FEA4 ^{59,69}	bZIP transcription factor	Promotes AG to repress WUS during stem cell termination in floral meristems, regulates shoot stem cell function??

Table 1