Published in final edited form as:

Curr Opin Plant Biol. 2012 December ; 15(6): 652–658. doi:10.1016/j.pbi.2012.05.007.

Argonautes compete for miR165/166 to regulate shoot apical meristem development

Zhonghui Zhang^{1,2} and Xiuren Zhang^{1,2,*}

¹Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843

²Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX 77843

Summary

Plant stem cells in the shoot apical meristem (SAM) possess the unique abilities of both self-renewal for SAM maintenance and providing undifferentiated daughter cells for initiation and subsequent development of aerial organs. The coordination between stem cell renewal and cell differentiation during organogenesis is regulated by elaborate genetic pathways involving numerous transcription factors and other molecules. In the past decade, microRNAs (miRNAs) have emerged as pivotal regulators in many biological processes including meristem homeostasis and differentiation in plants. In this review, we summarize current knowledge about the function and mechanism of a family of miRNAs (miR165/166), the miRNA-designated Argonautes (AGOs), and the miRNA-regulated targets in SAM development and maintenance.

Keywords

miRNA; Argonaute; HD-ZIP III transcriptional factors; shoot apical meristem; stem cell

Introduction

Stem cells are the ultimate source for organs and tissues in multi-cellular organisms. In plants, small populations of stem cells are confined within specialized niches that are located at the very tips of shoots called shoot apical meristems (SAMs). The stem cells in the SAM are maintained in a pluripotent state throughout the lifespan of the plants. As the stem cells divide, some daughter cells are retained within a small central zone in the SAMs for self-renewal, whereas the other daughter cells are dislodged to the periphery of the SAM for differentiation to form the aerial parts in higher plants: the shoot axis, side branches, leaves, and flowers. The delicate balance between self-renewal for meristem cells and continuous organogenesis are controlled by interwoven signaling pathways that involve miRNA-mediated regulation of gene expression through AGO effectors.

miRNAs are a group of small non-coding RNAs which are key regulators of virtually all biological processes in eukaryotes. miRNAs are processed by RNase III-like enzymes from imperfectly self-folded hairpin precursors. Mature miRNAs are incorporated into AGO-

© 2012 Elsevier Ltd. All rights reserved.

*Correspondence: xiuren.zhang@tamu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

containing RNA induced silencing complexes (RISCs) to repress the expression of targeted genes at a posttranscriptional level in a sequence-specific manner [1]. AGO proteins contain a variable N-terminal domain and conserved C-terminal PAZ, MID, and PIWI domains. The PAZ and MID domains recognize the 3' and 5' ends of miRNAs, respectively [2]. The PIWI domain has an RNaseH-like structure and carries out the endonuclease activity directed by miRNAs on complementary mRNA targets [3].

In the model organism *Arabidopsis thaliana*, approximately 300 miRNAs have been identified, and many of these miRNAs target transcription factors, indicating their critical regulatory roles in plant biology. The *Arabidopsis* genome also encodes nine functional AGO family members. AGO1 predominantly associates with small RNAs (sRNAs) with a 5' uridine (U) [4,5]. AGO1 is required to carry out the function of most miRNAs, and its inactivation severely impairs plant development, leading to pleiotropic phenotypes [6]. AGO2 preferentially binds to 21nt sRNAs with a 5' adenine (A) [4,7] and plays an important role in DNA repair [8] and host defense against viruses and bacteria [9–11]. AGO5 binds to sRNAs that have a 5' cytosine (C) [4], whereas AGO7 largely recruits a single miRNA, miR390, to elicit the biogenesis of trans-acting siRNAs [12]. Finally, AGO4, AGO6, and AGO9 all bind to 24nt sRNAs with a 5' A to mediate DNA methylation and epigenetic silencing with a partial functional redundancy [13,14].

AGO10, also known as PINHEAD and ZWILLE, is the closest homolog of AGO1 in *Arabidopsis*. AGO10 plays a critical role in SAM development, as plants with inactivation of the *AGO10* gene display empty apices or terminally differentiated organs in place of otherwise normal SAMs [15,16]. Interestingly, these SAM defects are only observed in the *Lansberg erecta* accession, but rarely in the Col-0 background, indicating an accession specific penetrance. In this review, we summarize recent progress in the study of the function and mechanism of AGO10/miR165/166/HD-ZIP III in meristem development and maintenance.

Role of the *HD-ZIP III* genes in stem cell activity

The *Arabidopsis* genome encodes five members of the class III homeodomain-leucine zipper (HD-ZIP III) transcription factors: *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*), *REVOLUTA* (*REV*), *CORONA* (*CNA*) and *ATHB8* [17]. In addition to the homeodomain DNA binding motif and leucine zipper dimerization domain, all HD-ZIP III transcription factors contain in their N termini, a START domain, which potentially binds a ligand, such as steroids or phospholipids. They also harbor in their C termini a Per-ARNT-Sim-like (PAS-like) MEKHLA domain, which has two functions: it can cause conformational changes in HD-ZIP III proteins and auto-inhibit the protein from dimerization; The MEKHLA domain is also a putative cellular sensor for light, oxygen, and redox potential signaling [18]. Upon perception of appropriate stimuli by the MEKHLA domain, the auto-inhibition of the HD-ZIP III dimerization is relieved so that the HD-ZIP III protein can bind to DNA for transcriptional activation [19•]. Phylogenetic analyses of the HD-ZIP III proteins place them into three sub-clades. *PHB* and *PHV* belong to a clade with 85% amino acid identity. Likewise, *CNA* and *ATHB8* are another closely-related pair with 75% amino acid identity. *REV* is in a separate clade, but shares approximately 60–66% amino acid identities with the other four members [20].

The *HD-ZIP III* family genes are well characterized as developmental regulators required for SAM establishment, vascular development, and polarity formation of lateral organs by promoting the adaxial identity [21]. Although loss-of-function mutants in four individual members in this family lack discernible phenotypic abnormalities, higher-order mutants such as *rev phb* and *rev phb phv* mutants fail to establish a SAM and often produce single pin-like

cotyledons [21,22]. On the other hand, gain-of-function *phb-D* and *rev-D* mutants result in enlarged meristems and adaxialized leaves [20,23]. Therefore, *PHB*, *PHV* and *REV* have overlapping functions in regulating SAM formation and leaf polarity. In contrast, *CNA* functions in parallel with the *CLAVATA* pathway to down-regulate *WUSHEL* (*WUS*) expression and thus confines the *WUS* activity in the stem cell niche [24]. Simultaneous loss of *PHB*, *PHV*, and *CNA* causes greatly enlarged SAMs with over-accumulated stem cells, suggesting that *CNA* and *REV* act antagonistically with each other in embryonic development [21,25].

The *HD-ZIP III* genes also regulate post-embryonic development. Loss-of-function *rev* mutants frequently display incomplete or abortive structures at the positions of lateral organs [22, 26], indicating that *REV* promotes initiation and development of auxiliary meristems. Additional loss of *PHB* or *PHV* enhances *rev* phenotypes. *ATHB8* plays a role in vascular development [27] and acts redundantly with *CNA* to suppress axillary and floral meristem defects of *rev* mutants, indicating that the *HD-ZIP III* genes play both redundant and antagonistic roles in post-embryonic meristem activities [21].

Regulation of HD-ZIP III activities

As the *HD-ZIP III* genes are critical for meristem development, the precise regulation of their activity is essential. HD-ZIP III activity can be modulated at a posttranslational level by a peptide ligand known as the LITTLE ZIPPER proteins (ZPR) [28,29]. ZPRs are a group of small proteins, 67~72 amino acids in length, containing the ZIP motifs in their central regions, but lacking DNA-binding domains. ZPRs form non-functional heterodimers with the HD-ZIP III proteins and prevent their transcription factor activity. Failure to repress HD-ZIP III activity, as in the *zpr3 zpr4* double mutants, causes formation of an enlarged SAM [28]. Interestingly, the *HD-ZIP III* genes can positively regulate the transcription of *ZPRs*, forming a negative feedback loop in regulating stem cell activity [28,29].

Regulation of the *HD-ZIP III* genes can occur at the posttranscriptional level through miR165/166. The Arabidopsis genome harbors two *MIR165* loci (*a* and *b*) and seven *MIR166* loci (*a to g*), which encode two structurally similar miRNAs that differ in sequences by only one single nucleotide. miR165/166 have nearly perfect complementarity with sequence located within junction of the 3' end of the fourth exon and the 5' end of the fifth exon of *HD-ZIP III* transcripts [30]. Mature miR165/166 accumulates throughout embryos during their early globular stages, but in subsequent developmental stages, they are spatially restricted in the abaxial domains of cotyledons and leaf primordia [31,32]. Consequently, the abaxial restriction of miR165/166 establishes the spatial expression pattern of the *HD-ZIP III* genes, which are limited to the adaxial sides of leaf primordia [25,31]. This complementary pattern of expression for miR165/166 and their targeted genes along the ab/adaxial axis of cotyledons and leaf primordia provides a spatial cue for proper SAM development, because new meristems reside entirely within the adaxial domain defined by *HD-ZIP III* expression and are excluded from the abaxial domain marked by miR165/166 [33].

Several gain-of-function *phv-D*, *phb-D* and *rev-D* mutants have been recovered, and interestingly, all the mutations identified thus far occur within the complementary sites for miR165/166. These mutations prevent the *HD-ZIP III* genes from miR165/166-guided repression leading to ectopic accumulation of the transcripts in abaxial domains of leaf primordia. As a result, these mutants confer enlarged meristems and radial, adaxialized leaves [30,34]. In contrast, two semi-dominant mutants of miR166, *jabba-1D* (*jab-1D*) [25] and *meristem enlarged 1* (*men1*) [35] have elevated levels of miR166g and miR166a, respectively, leading to down-regulation of all *HD-ZIP III* genes, except *REV*, in the SAM.

REV is not repressed in these mutants likely because the expression domains of miR166a and miR166g have no or only limited overlap with those of *REV*. *jab-1D* and *men1* exhibit enlarged SAM and abaxialization of lateral organs, reminiscent of loss-of-function *phb phv cna* mutants. In addition, overexpression of miR165/166 driven by a constitutive 35S promoter causes a drastic reduction in the transcript levels of all five *HD-ZIP III* genes in *Arabidopsis* and a range of phenotypes from enlarged SAM to loss of SAM, depending on biological contexts [36,37].

Sorting of miR165/166 into AGO1 and AGO10

miR165/166 regulate *HD-ZIP III* transcripts through AGO proteins. Because AGO1 is required for the function of most miRNAs including miR165/166, it represses the *HD-ZIP III* transcripts in the abaxial domains of organs where miR165/166 accumulate [6,38]. Some of the *ago1* mutant phenotypes superficially resemble those of *ago10* mutants. For instance, approximately 10% of the null *ago1* seedlings display a defective SAM [39]. Double mutants of strong *ago1* and *ago10* alleles result in embryonic lethality, leading to an earlier proposal that AGO1 and AGO10 have redundant function during SAM development, presumably through binding a common set of miRNAs. However, genetic studies suggest that their roles in miRNA- and transgene-mediated posttranscriptional gene silencing pathways are different [•40]. Promoter-swapping experiments revealed that *AGO1* and *AGO10* genes cannot substitute for each other [•40,•41]. Moreover, loss of the *AGO10* gene reestablishes both small-interfering RNA and miRNA pathways for a subset of AGO1-targeted genes in the hypomorphic *ago1* alleles indicating that *AGO10* is a negative regulator of *AGO1* [•40].

Recently, Liu and colleagues observed an abnormally elevated abundance of miR165/166 in embryonic SAMs and leaves in *ago10* mutants, providing the first evidence of biological interaction between *AGO10* and miR165/166 [•31]. They also found that the aberrant *ago10* phenotypes are partially rescued by increasing levels of *HD-ZIP III* transcripts either by expressing miR165/166 resistant forms of *HD-ZIP III* transgenes or decreasing levels of miR165/166 in the SAM and leaf by target mimicry [•31]. These results elegantly demonstrate that increased miR165/166 levels downregulate *HD-ZIP III* transcripts in *ago10* mutant, accounting for defects of SAM and leaf polarity.

Zhu and colleagues set out to clarify AGO10 function and mechanism by identifying AGO10-bound sRNAs [•41]. They found that AGO10 behaves very differently from other AGOs. It has a narrow preference spectrum and predominantly associates with miR165/166 [•41]. All miR165/166 family members adopt distinct structural features in their miRNA/RNA* (miRNA/*) duplexes that are absent from other miRNAs. Zhu et al explored whether the unique structures of the miRNA/* play a role in their specific sorting into AGO10 by expressing miR166/* duplexes in heterogeneous precursors. They observed that the loading of miR166 into AGO10 is compromised if the miR166/* structures are disrupted in the chimeric precursors, whereas the loading is not affected if the intact miR166/* structures are maintained in the chimeric precursors. On the other hand, AGO1 is more tolerant for the modifications in the miRNA/* structures [•41]. In summary, the highly conserved secondary structures in the miRNA/* duplexes account for their specific preference of AGO10 for miR165/166 [•41]. This finding is in line with previous observations in flies and worms that routing of sRNAs into different AGOs depends on the structures of the sRNA duplexes [42–44].

AGO10 competes with AGO1 for miR165/166 to regulate meristem development

Because AGO proteins mainly function as repressors for miRNA-targeted genes, it could be generally thought that AGO10 acts in a similar way by specifically recruiting miR165/166 to downregulate the *HD-ZIP III* transcripts. Indeed, AGO10 possesses the catalytic activity *in vitro* and is capable of slicing the *HD-ZIP III* transcripts in RISC reconstitution assays [•41,•45]. However, this activity is dispensable for the miR165/166-dependent AGO10 function in plants because slicing-inactivated AGO10 can rescue *ago10* mutants as efficiently as wild-type AGO10. Moreover, AGO10 is a positive regulator of *HD-ZIP III* genes *in vivo* as transcript levels of all *HD-ZIP III* genes are reduced in *ago10* mutants compared to those in wild-type plants. This can be further demonstrated by the coinciding presence of AGO10 and the *HD-ZIP III* transcripts in the adaxial sides of embryos and leaf primordia [23,26,•46].

AGO10 was proposed to participate in translational inhibition for numerous miRNA target genes [•40,47]. Under this model, AGO10 might regulate the *HD-ZIP III* genes through translational repression. Ji and colleagues addresses this issue using *ZPR3*, which is a direct target of PHB, as a proxy reporter for the HD-ZIP III proteins [•45]. *ZPR3* levels are increased in the miRNA165/166-resistant *HD-ZIP III* mutants. By contrast, *ZPR3* accumulation is decreased in the loss-of-function *HD-ZIP III* mutants [29] and in the *ago10* mutants. These studies indicate that levels of the HD-ZIP III proteins are lower in *ago10* than in wild-type plants, which further suggests that AGO10-miR165/166 does not inhibit the translation of the HD-ZIP III proteins. Moreover, recent studies in animal systems demonstrate that miRNA-primed translational repression occurs at the initial stage of RNA silencing and is subsequently followed by mRNA decay [•48,•49]. Given that elevated *HD-ZIP III* transcripts are observed in *ago10* mutants, translational repression is unlikely to be the mechanism for the AGO10-miR165/166 complex.

What does AGO10/miR165/166 do *in vivo*? Liu et al. have shown that AGO10 modulates SAM maintenance and establishment of organ polarity by genetically repressing expression of miR165/166 [•31]. In *ago10* mutants, ectopically accumulated miR165/166 is re-sorted into AGO1 [•41], which is the principal slicer in plants, leading to a decrease in *HD-ZIP III* transcripts in the AGO10 expression domains. This alteration of *HD-ZIP III* accumulation underlies the defects of SAM maintenance and organ polarity of *ago10* mutants. Because simple sequestration of miR165/166 can rescue the *ago10* phenotype, it is possible that the primary function of AGO10 is to antagonize miR165/166 activity by preventing it from being loaded into the catalytically potent AGO1 and subsequently targeting *HD-ZIP III* transcripts. In support of this idea, transgenic plants expressing a variant version of miR166/* that can be recruited by the more promiscuous AGO1, but not by AGO10, indeed mimic *ago10* mutant phenotypes [•41]. Furthermore, AGO10 demonstrates stronger binding capability for miR166 than does AGO1 in competition binding assays and, thus, does possess a decoying ability for these miRNAs [•41].

AGO10 is expressed throughout the SAMs, the vasculature primordia, and the adaxial domains but not in the abaxial sides of leaf primordia. This expression pattern is essentially the same for that of the *HD-ZIP III* transcripts (Figure 1). Tucker et al previously reported that AGO10 is engaged in non-cell autonomous communication between the meristem and the nascent vasculature underneath to promote SAM maintenance and organ polarity [•46]. AGO10 itself is unlikely to move, whereas miR165/166 can move between cells [50, 51]. Thus it is tempting to believe that the “apparent” non-cell autonomous nature of AGO10 results from its associated miR165/166. Under this model, AGO10 can specifically entrap miR165/166, which may diffuse from the abaxial regions of developing leaf primordia, in

the vasculature and prevent the influx of these miRNAs into the meristem above (Figure 1). Meanwhile, AGO10 may also sequester the residue miR165/166 present in the SAMs and adaxial domains of leaf primordia, and trigger their decay through a yet identified mechanism. As a result, the higher accumulation of *HD-ZIP III* transcripts in the SAMs and the adaxial regions of leaf primordia provides a correct spatial cue for the SAM development and leaf polarity (Figure 1).

During post-embryonic development, AGO10 continues to regulate meristem activity and organ polarity, but its role is more complicated. Although AGO10 promotes axillary meristem formation [52], it stimulates the termination of floral stem cells, as suggested by *ago10* loss-of-function mutants, which result in prolonged floral meristem maintenance and, consequently, an enlargement of inflorescence [15,16]. Similar to its role in SAM development, AGO10 attenuates miR165/166 activity to terminate floral meristems as overexpression of miR166 [25] and higher-order loss-of-function *HD-ZIP III* mutants [21,45] phenocopy *ago10* mutants. Intriguingly, AGO1 also promotes floral determinacy, but this regulation is through miR165/166-mediated repression of the *HD-ZIP III* transcripts, a mechanism different from that of AGO10. Therefore, mis-expression of the *HD-ZIP III* genes by rendering them resistant to miR165/166 can result in prolonged floral stem cell activity [45]. This observation is reminiscent of the situation in SAM development: although sequestering miR165/166 from the region of AGO10 expression rescues *ago10* mutants, attenuation of miR165/166 activity throughout the AGO1 region of expression accelerates the *ago10* mutant phenotype [41] (Figure 1). In summary, in both SAM and floral stem cell development, the *HD-ZIP III* activity should be maintained in a proper gradient to assure the balance between stem-cell renewal and differentiation with higher levels of *HD-ZIP* activity in the region of AGO10 expression and lower levels outside this region.

Conclusion and Perspective

Recent discoveries have provided new and exciting data that enable us to better understand the molecular mechanism underlying AGO10 regulation of stem cell activity. Although old puzzles have been solved, many new questions have been raised. First, how did AGO10 evolve to specifically recognize the relevant structural features of miR165/166 duplexes, whereas AGO1 is less discriminating for miRNA/* structure? Second, given that AGO10 specifically recruits miR165/166, why does AGO10 not cleave the target *HD-ZIP III* transcripts *in vivo*? Third, how does AGO10 repress the expression of miR165/166 levels in planta? Fourth, is AGO10-miR165/166 interaction a unique case for AGO-mediated attenuation of miRNA activity? Finally, RNA transcripts can crosstalk with each other by competing for common miRNAs in animals with miRNA response elements as the foundation of this interaction [53,54]. In Arabidopsis, *REV* and *ATHB8/CNA* act somehow antagonistically in stem cell development in an unknown manner. Given that they all contain miR165/166-targeting sequences and the AGO10-miR165/166 complex is an unproductive RISC, do *REV* and *ATHB8/CNA* cross-regulate with each other through shared miR165/166? Certainly all these questions and many others will be addressed with the collaborative efforts from our active research communities in the RNAi and stem cell fields in the near future.

Acknowledgments

Our work is supported by the National Science Foundation (MCB-0951120), NIH (R21AI097570) and Welch foundation (A-1777). The authors thank Drs. Hai Huang, Diana Dugas, Timothy Devarenne and anonymous reviewers for their critical reading of the manuscript. We apologize to colleagues whose research we failed to adequately cite due to space limitations.

References and recommended reading

Papers of particular interest have been highlighted as:

- of special interest
- of outstanding interest

1. Czech B, Hannon GJ. Small RNA sorting: matchmaking for Argonautes. *Nat Rev Genet.* 2011; 12:19–31. [PubMed: 21116305]
2. Wang Y, Juranek S, Li H, Sheng G, Wardle GS, Tuschl T, Patel DJ. Nucleation, propagation and cleavage of target RNAs in Ago silencing complexes. *Nature.* 2009; 461:754–761. [PubMed: 19812667]
3. Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song JJ, Hammond SM, Joshua-Tor L, Hannon GJ. Argonaute2 is the catalytic engine of mammalian RNAi. *Science.* 2004; 305:1437–1441. [PubMed: 15284456]
4. Mi S, Cai T, Hu Y, Chen Y, Hodges E, Ni F, Wu L, Li S, Zhou H, Long C, et al. Sorting of small RNAs into Arabidopsis argonaute complexes is directed by the 5' terminal nucleotide. *Cell.* 2008; 133:116–127. [PubMed: 18342361]
5. Wang H, Zhang X, Liu J, Kiba T, Woo J, Ojo T, Hafner M, Tuschl T, Chua NH, Wang XJ. Deep sequencing of small RNAs specifically associated with Arabidopsis AGO1 and AGO4 uncovers new AGO functions. *Plant J.* 2011; 67:292–304. [PubMed: 21457371]
6. Mallory A, Vaucheret H. Form, function, and regulation of ARGONAUTE proteins. *Plant Cell.* 2010; 22:3879–3889. [PubMed: 21183704]
7. Takeda A, Iwasaki S, Watanabe T, Utsumi M, Watanabe Y. The mechanism selecting the guide strand from small RNA duplexes is different among argonaute proteins. *Plant Cell Physiol.* 2008; 49:493–500. [PubMed: 18344228]
8. Wei W, Ba Z, Gao M, Wu Y, Ma Y, Amiard S, White CI, Danielsen JM, Yang YG, Qi Y. A Role for Small RNAs in DNA Double-Strand Break Repair. *Cell.* 2012; 149:101–112. [PubMed: 22445173]
9. Wang XB, Jovel J, Udomporn P, Wang Y, Wu Q, Li WX, Gascoli V, Vaucheret H, Ding SW. The 21-nucleotide, but not 22-nucleotide, viral secondary small interfering RNAs direct potent antiviral defense by two cooperative argonautes in Arabidopsis thaliana. *Plant Cell.* 2011; 23:1625–1638. [PubMed: 21467580]
10. Zhang X, Zhao H, Gao S, Wang WC, Katiyar-Agarwal S, Huang HD, Raikhel N, Jin H. Arabidopsis Argonaute 2 regulates innate immunity via miRNA393(*)-mediated silencing of a Golgi-localized SNARE gene, MEMB12. *Mol Cell.* 2011; 42:356–366. [PubMed: 21549312]
11. Harvey JJ, Lewsey MG, Patel K, Westwood J, Heimstadt S, Carr JP, Baulcombe DC. An antiviral defense role of AGO2 in plants. *PLoS One.* 2011; 6:e14639. [PubMed: 21305057]
12. Montgomery TA, Howell MD, Cuperus JT, Li D, Hansen JE, Alexander AL, Chapman EJ, Fahlgren N, Allen E, Carrington JC. Specificity of ARGONAUTE7-miR390 interaction and dual functionality in TAS3 trans-acting siRNA formation. *Cell.* 2008; 133:128–141. [PubMed: 18342362]
13. Havecker ER, Wallbridge LM, Hardcastle TJ, Bush MS, Kelly KA, Dunn RM, Schwach F, Doonan JH, Baulcombe DC. The Arabidopsis RNA-directed DNA methylation argonautes functionally diverge based on their expression and interaction with target loci. *Plant Cell.* 2010; 22:321–334. [PubMed: 20173091]
14. Olmedo-Monfil V, Duran-Figueroa N, Arteaga-Vazquez M, Demesa-Arevalo E, Autran D, Grimanelli D, Slotkin RK, Martienssen RA, Vielle-Calzada JP. Control of female gamete formation by a small RNA pathway in Arabidopsis. *Nature.* 464:628–632. [PubMed: 20208518]
15. Lynn K, Fernandez A, Aida M, Sedbrook J, Tasaka M, Masson P, Barton MK. The PINHEAD/ZWILLE gene acts pleiotropically in Arabidopsis development and has overlapping functions with the ARGONAUTE1 gene. *Development.* 1999; 126:469–481. [PubMed: 9876176]

16. Moussian B, Schoof H, Haecker A, Jurgens G, Laux T. Role of the ZWILLE gene in the regulation of central shoot meristem cell fate during Arabidopsis embryogenesis. *Embo J.* 1998; 17:1799–1809. [PubMed: 9501101]
17. Ariel FD, Manavella PA, Dezar CA, Chan RL. The true story of the HD-Zip family. *Trends Plant Sci.* 2007; 12:419–426. [PubMed: 17698401]
18. Mukherjee K, Burglin TR. MEKHLA, a novel domain with similarity to PAS domains, is fused to plant homeodomain-leucine zipper III proteins. *Plant Physiol.* 2006; 140:1142–1150. [PubMed: 16607028]
19. Magnani E, Barton MK. 2011. A per-ARNT-sim-like sensor domain uniquely regulates the activity of the homeodomain leucine zipper transcription factor REVOLUTA in Arabidopsis. *Plant Cell.* 23:567–582. This paper shows that MEKHLA domain of REVOLUTA inhibits the protein self-dimerization through a sequence-independent steric masking mechanism. MEKHLA-mediated self-inhibition is relieved upon its perception of a yet identified cellular signal. [PubMed: 21357492]
20. Byrne ME. Shoot meristem function and leaf polarity: the role of class III HD-ZIP genes. *PLoS Genet.* 2006; 2:e89. [PubMed: 16846251]
21. Prigge MJ, Otsuga D, Alonso JM, Ecker JR, Drews GN, Clark SE. Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in Arabidopsis development. *Plant Cell.* 2005; 17:61–76. [PubMed: 15598805]
22. Talbert PB, Adler HT, Parks DW, Comai L. The REVOLUTA gene is necessary for apical meristem development and for limiting cell divisions in the leaves and stems of Arabidopsis thaliana. *Development.* 1995; 121:2723–2735. [PubMed: 7555701]
23. McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK. Role of PHABULOSA and PHAVOLUTA in determining radial patterning in shoots. *Nature.* 2001; 411:709–713. [PubMed: 11395776]
24. Green KA, Prigge MJ, Katzman RB, Clark SE. CORONA, a member of the class III homeodomain leucine zipper gene family in Arabidopsis, regulates stem cell specification and organogenesis. *Plant Cell.* 2005; 17:691–704. [PubMed: 15705957]
25. Williams L, Grigg SP, Xie M, Christensen S, Fletcher JC. Regulation of Arabidopsis shoot apical meristem and lateral organ formation by microRNA miR166g and its AtHD-ZIP target genes. *Development.* 2005; 132:3657–3668. [PubMed: 16033795]
26. Otsuga D, DeGuzman B, Prigge MJ, Drews GN, Clark SE. REVOLUTA regulates meristem initiation at lateral positions. *Plant J.* 2001; 25:223–236. [PubMed: 11169198]
27. Baima S, Possenti M, Matteucci A, Wisman E, Altamura MM, Ruberti I, Morelli G. The arabidopsis ATHB-8 HD-zip protein acts as a differentiation-promoting transcription factor of the vascular meristems. *Plant Physiol.* 2001; 126:643–655. [PubMed: 11402194]
28. Kim YS, Kim SG, Lee M, Lee I, Park HY, Seo PJ, Jung JH, Kwon EJ, Suh SW, Paek KH, et al. HD-ZIP III activity is modulated by competitive inhibitors via a feedback loop in Arabidopsis shoot apical meristem development. *Plant Cell.* 2008; 20:920–933. [PubMed: 18408069]
29. Wenkel S, Emery J, Hou BH, Evans MM, Barton MK. A feedback regulatory module formed by LITTLE ZIPPER and HD-ZIP III genes. *Plant Cell.* 2007; 19:3379–3390. [PubMed: 18055602]
30. Mallory AC, Reinhart BJ, Jones-Rhoades MW, Tang G, Zamore PD, Barton MK, Bartel DP. MicroRNA control of PHABULOSA in leaf development: importance of pairing to the microRNA 5' region. *Embo J.* 2004; 23:3356–3364. [PubMed: 15282547]
31. Liu Q, Yao X, Pi L, Wang H, Cui X, Huang H. The ARGONAUTE10 gene modulates shoot apical meristem maintenance and establishment of leaf polarity by repressing miR165/166 in Arabidopsis. *Plant J.* 2009; 58:27–40. In this study, the authors have combined genetic and histological analyses to show that AGO10 regulates SAM development and leaf polarity by genetically repressing miR165/166. For the first time, the authors proposed that ectopically elevated miR165/166 accumulation in embryonic SAM and leaf causes reduction of HD-ZIP III transcripts in *ago10* mutants, leading to defective SAM and leaf polarity. [PubMed: 19054365]
32. Yao X, Wang H, Li H, Yuan Z, Li F, Yang L, Huang H. Two types of cis-acting elements control the abaxial epidermis-specific transcription of the MIR165a and MIR166a genes. *FEBS Lett.* 2009; 583:3711–3717. [PubMed: 19879265]

33. Barton MK. Twenty years on: the inner workings of the shoot apical meristem, a developmental dynamo. *Dev Biol.* 2010; 341:95–113. [PubMed: 19961843]
34. Emery JF, Floyd SK, Alvarez J, Eshed Y, Hawker NP, Izhaki A, Baum SF, Bowman JL. Radial patterning of Arabidopsis shoots by class III HD-ZIP and KANADI genes. *Curr Biol.* 2003; 13:1768–1774. [PubMed: 14561401]
35. Kim J, Jung JH, Reyes JL, Kim YS, Kim SY, Chung KS, Kim JA, Lee M, Lee Y, Narry Kim V, et al. microRNA-directed cleavage of ATHB15 mRNA regulates vascular development in Arabidopsis inflorescence stems. *Plant J.* 2005; 42:84–94. [PubMed: 15773855]
36. Zhou GK, Kubo M, Zhong R, Demura T, Ye ZH. Overexpression of miR165 affects apical meristem formation, organ polarity establishment and vascular development in Arabidopsis. *Plant Cell Physiol.* 2007; 48:391–404. [PubMed: 17237362]
37. Jung JH, Park CM. MIR166/165 genes exhibit dynamic expression patterns in regulating shoot apical meristem and floral development in Arabidopsis. *Planta.* 2007; 225:1327–1338. [PubMed: 17109148]
38. Kidner CA, Martienssen RA. Spatially restricted microRNA directs leaf polarity through ARGONAUTE1. *Nature.* 2004; 428:81–84. [PubMed: 14999284]
39. Kidner CA, Martienssen RA. The role of ARGONAUTE1 (AGO1) in meristem formation and identity. *Dev Biol.* 2005; 280:504–517. [PubMed: 15882589]
40. Mallory AC, Hinze A, Tucker MR, Bouche N, Gasciolli V, Elmayan T, Laussergues D, Jauvion V, Vaucheret H, Laux T. Redundant and specific roles of the ARGONAUTE proteins AGO1 and ZLL in development and small RNA-directed gene silencing. *PLoS Genet.* 2009; 5:e1000646. By using promoter and protein domain-swapping assays and some markers of RNA silencing pathways, the author discovered that AGO10 suppresses a subset of sRNA-directed gene regulatory defects of weak *ago1* mutations. The authors also identified that all domains, except PAZ, are not interchangeable between AGO1 and AGO10 for their functional specificities. [PubMed: 19763164]
41. Zhu H, Hu F, Wang R, Zhou X, Sze SH, Liou LW, Barefoot A, Dickman M, Zhang X. Arabidopsis Argonaute10 specifically sequesters miR166/165 to regulate shoot apical meristem development. *Cell.* 2011; 145:242–256. By using combinational biochemical and genetic analyses, the authors discovered that AGO10 specifically interacts with miR165/166. The specific association is determined by a distinct structure of the miR165/166 duplex. The authors further show that deficient loading of miR165/166 into AGO10 results in the pinhead phenotype. They also found that the miRNA binding ability of AGO10, but not its catalytic activity, is required for SAM development, and AGO10 has a higher binding affinity for miR166 than does AGO1, a principal contributor to miRNA-mediated silencing. AGO10 is proposed to sequester miR165/166 to maintain the SAM by antagonizing the activities of AGO1. [PubMed: 21496644]
42. Tomari Y, Du T, Zamore PD. Sorting of Drosophila small silencing RNAs. *Cell.* 2007; 130:299–308. [PubMed: 17662944]
43. Forstemann K, Horwich MD, Wee L, Tomari Y, Zamore PD. Drosophila microRNAs are sorted into functionally distinct argonaute complexes after production by dicer-1. *Cell.* 2007; 130:287–297. [PubMed: 17662943]
44. Steiner FA, Hoogstrate SW, Okihara KL, Thijssen KL, Ketting RF, Plasterk RH, Sijen T. Structural features of small RNA precursors determine Argonaute loading in *Caenorhabditis elegans*. *Nat Struct Mol Biol.* 2007; 14:927–933. [PubMed: 17891148]
45. Ji L, Liu X, Yan J, Wang W, Yumul RE, Kim YJ, Dinh TT, Liu J, Cui X, Zheng B, et al. ARGONAUTE10 and ARGONAUTE1 regulate the termination of floral stem cells through two microRNAs in Arabidopsis. *PLoS Genet.* 2011; 7:e1001358. By using a series of genetic screening and analyses, the authors demonstrated that AGO10 and HD-ZIP III are involved in floral stem cell termination. They also discovered that AGO10, just like AGO1, is an active “slicer” in vitro. But they promote floral stem cell termination by exerting opposite effects on miR165/166 in vivo. [PubMed: 21483759]
46. Tucker MR, Hinze A, Tucker EJ, Takada S, Jurgens G, Laux T. Vascular signalling mediated by ZWILLE potentiates WUSCHEL function during shoot meristem stem cell development in the Arabidopsis embryo. *Development.* 2008; 135:2839–2843. In this study, by using mosaic genetic assays, the authors discovered that AGO10 acts to maintain the stem cells from the neighbouring

vascular primordium indicating a non-cell-autonomous nature of AGO10 function. As AGO10 itself may not move between cells, this study suggests there is a yet identified mobile signal emanated from AGO10 protein. This signal is likely miR165/166 identified in the study by Zhu, *et al* (ref 41). [PubMed: 18653559]

47. Brodersen P, Sakvarelidze-Achard L, Bruun-Rasmussen M, Dunoyer P, Yamamoto YY, Sieburth L, Voinnet O. Widespread translational inhibition by plant miRNAs and siRNAs. *Science*. 2008; 320:1185–1190. [PubMed: 18483398]
48. Bazzini AA, Lee MT, Giraldez AJ. Ribosome profiling shows that miR-430 reduces translation before causing mRNA decay in zebrafish. *Science*. 2012; 336:233–237. [PubMed: 22422859]
49. Djuranovic S, Nahvi A, Green R. miRNA-mediated gene silencing by translational repression followed by mRNA deadenylation and decay. *Science*. 2012; 336:237–240. These two papers carefully investigated RNA-silencing mechanisms and found that miRNA-primed translational repression occurs earlier than mRNA decay. These studies, although not performed on *Arabidopsis*, revoke our reconsideration whether or not there is miRNA-mediated translational inhibition without observable change of the steady-state levels in mRNAs in plants. [PubMed: 22499947]
50. Carlsbecker A, Lee JY, Roberts CJ, Dettmer J, Lehesranta S, Zhou J, Lindgren O, Moreno-Risueno MA, Vaten A, Thitamadee S, et al. Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature*. 2010; 465:316–321. [PubMed: 20410882]
51. Chitwood DH, Timmermans MC. Small RNAs are on the move. *Nature*. 467:415–419. [PubMed: 20864994]
52. McConnell JR, Barton MK. Effect of mutations in the pinhead gene of *Arabidopsis* on the formation of shoot apical meristems. *Developmental Genetics*. 1995; 16:358–366.
53. Tay Y, Kats L, Salmena L, Weiss D, Tan S, Ala U, Karreth F, Poliseno L, Provero P, Cunto F, et al. Coding-Independent Regulation of the Tumor Suppressor PTEN by Competing Endogenous mRNAs. *Cell*. 2011; 147:344–357. [PubMed: 22000013]
54. Rubio-Somoza I, Weigel D, Franco-Zorilla J, García J, Paz-Ares J. ceRNAs: miRNA Target Mimic Mimics. *Cell*. 2011; 147:143.

Highlights

SAM is the ultimate source aerial organs

HD-ZIP III genes positively regulate SAM development

miR165/166 target *HD-ZIP III* transcripts

Both AGO1 and AGO10 bind to miR165/166 to regulate *HD-ZIP III* transcripts, but their modes of action are opposite

AGO10 competes with AGO1 for miR165/166 to regulate meristem development

\$watermark-text

\$watermark-text

\$watermark-text

Figure 1.

AGO10 is expressed at moderate levels throughout the SAM and in the adaxial domains of leaf primordia and at higher levels in the vasculature precursors, whereas *AGO1* accumulates ubiquitously in all tissues and organs. The presence of the *HD-ZIP III* transcripts essentially coincides with that of *AGO10* in the SAM and the adaxial leaf domains and is complementary to that of miR165/166 in the abaxial domain. *AGO10* competes with *AGO1* for miR165/166 to protect the *HD-ZIP III* transcripts from post-transcriptional regulation, thereby promoting meristem activities. Under this model, *AGO10* may entrap miR165/166 in certain regions (grey solid arrow) or trigger their degradation by a yet identified mechanism (grey dotted arrow). SAM, shoot apical meristem. P0 and P1, newly formed leaf primordia. Solid green arrow and red block lines show positive and negative regulation, respectively. Orange and blue color separated by black dotted lines shows the expression domains of miR165/166 and their targeted *HD-ZIP III* transcripts, respectively. Yellow lines, although labeled miR166, are representative of both miR165 and miR166 expression.