Published in final edited form as:

Curr Opin Plant Biol. 2016 February ; 29: 64–72. doi:10.1016/j.pbi.2015.11.006.

The role of small RNAs in vegetative shoot development

Jim P. Fouracre and R. Scott Poethig

Biology Department, University of Pennsylvania, 433 S. University Ave, Philadelphia, Pennsylvania, 19104, USA

Abstract

Shoot development consists of the production of lateral organs in predictable spatial and temporal patterns at the shoot apex. To properly integrate such programs of growth across different cell and tissue types, plants require highly complex and robust genetic networks. Over the last twenty years, the roles of small, non-coding RNAs (sRNAs) in these networks have become increasingly apparent, not least in vegetative shoot growth. In this review, we describe recent progress in understanding the contribution of sRNAs to the regulation of vegetative shoot growth, and outline persisting experimental limitations in the field.

Keywords

miRNA; ta-siRNA; leaf development; shoot apical meristem

Shoot development consists of the production of lateral organs in predictable spatial and temporal patterns at the shoot apex. To properly integrate such programs of growth across different cell and tissue types, plants require highly complex and robust genetic networks. Over the last twenty years, the roles of small, non-coding RNAs (sRNAs) in these networks have become increasingly apparent, not least in vegetative shoot growth. In this review, we describe recent progress in understanding the contribution of sRNAs to the regulation of vegetative shoot growth, and outline persisting experimental limitations in the field.

Small RNAs enhance the complexity of developmental pathways

sRNAs are 20–25 nucleotide transcripts that are derived from longer double-stranded RNA (dsRNA) precursors (consisting of either two separate strands of RNA or a single-stranded hairpin) by the endonuclease activity of DICER-like (DCL) proteins. They function by binding to target transcripts via sequence complementarity, and can repress gene expression at either a transcriptional or post-transcriptional level. The mechanism of repression depends on the class of sRNA but, in all cases, silencing complexes require an ARGONAUTE (AGO) family protein [1,2].

Corresponding author: Poethig, R. S. (spoethig@sas.upenn.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.

A number of features permit sRNAs to augment protein-based control mechanisms. These include potentially enhanced intercellular mobility due to their small size, the ability to act as repressors at multiple levels of gene expression, specific base-pairing interactions with target genes, and the capacity to repress a number of targets simultaneously. The classification, biogenesis, silencing mechanisms, genetic networks, and mobility of sRNAs have been discussed in detail in a number of recent reviews [1–7]. Here we provide an update of our understanding of the roles of two particularly important classes of sRNA — microRNAs (miRNAs) and trans-acting small interfering RNAs (ta-siRNAs) [5] — in shoot development. Following a discussion of recent studies, we conclude by outlining some important gaps in our understanding of the mechanisms by which sRNAs operate to regulate gene expression in plants.

Initiation and maintenance of the shoot apical meristem

The correct organization and maintenance of the shoot apical meristem (SAM) is fundamental to vegetative growth. The SAM is specified early in embryonic development, and consists of the pool of stem cells from which all above ground organs are derived, and the surrounding region of undifferentiated meristematic cells [8]. Plants that lack a functional SAM produce morphologically normal embryos, but are unable to produce a shoot [9]. One of the first clues that sRNAs are important for plant development came from the identification of a dominant allele of the *Arabidopsis* class III HOMEODOMAIN LEUCINE ZIPPER (HD-ZIP III) transcription factor, *PHABULOSA* (*PHB*), which perturbed leaf polarity and caused meristems to be produced ectopically on leaves [10]. This allele, and a similar mutation in the related gene *PHAVOLUTA* (*PHV*) [11], were later demonstrated to encode transcripts resistant to cleavage by miRNA165/166 [12,13]. Consistent with the phenotype of these gain-of-function mutations, plants triply mutant for *phb*, *phv* and the related gene, *revoluta* (*rev*), are unable to produce a SAM [14,15]. Loss-of-function mutations in the *AGO* family member, *PINHEAD/ZWILLE/AGO10*, produce a similar aborted embryonic SAM phenotype [16] as a consequence of an increase in the activity of miRNA165/miR166 and a concomitant reduction of HD-ZIP III expression [17,18] (Figure 1A). *AGO10* acts by sequestering members of the miRNA165/166 family, thus preventing them from interacting with *AGO1* [19,20], the primary mediator of miRNA-mediated gene repression in *Arabidopsis*.

NO APICAL MERISTEM/CUP SHAPED COTYLEDON (*NAC*) genes specify the boundary between the meristem and the surrounding cotyledons [21]. Plants doubly mutant for two of these genes, *cuc1 cuc2*, have fused cotyledons and completely lack a SAM [22]. *CUC1* and *CUC2* are targets of miRNA164 [23]. Although constitutive expression of *miR164* produces a shoot meristemless phenotype reminiscent of *cuc1 cuc2* double mutants [23], plants expressing a miR164-resistant version of *CUC1* [24] or *CUC2* [23], as well as plants with severely reduced miR164 activity [25], have defects in leaf serration but otherwise appear quite normal during the vegetative phase of shoot development. These results suggest that whereas *CUC1* and *CUC2* are essential for the initiation of the SAM, miR164 regulation of *CUC1* and *CUC2* is not important for the subsequent maintenance of the SAM during vegetative development.

Recent work has revealed a role for miR394 in this process [26**]. The SAM is divided into three layers (L1-L3). The transcription factor, *WUSCHEL* (*WUS*), is expressed in a small group of cells in the innermost (L3) layer, where it promotes the proliferation of these and surrounding cells in a feedback loop with *CLAVATA* (*CLV*) genes [27]. *miR394* is expressed in the outermost protoderm (L1) layer, from which it diffuses to produce a gradient of expression that overlaps with the expression domain of its target, the F-Box protein LEAF CURLING RESPONSIVENESS (*LCR*) [26**] (Figure 1A). The phenotype of plants deficient for miR394, and the phenotype of plants expressing miR394-resistant *LCR* alleles, demonstrates that repression of *LCR* by miR394 is essential for stem cell proliferation, and that the maintenance of the embryonic SAM requires convergent intercellular movement of both *WUS* and miR394 (26**). The recent demonstration that reduced HAIRY MERISTEM family activity, via expression of the regulator miR171 under the *WUS* promoter, leads to premature vegetative meristem termination [28] suggests that multiple miRNAs may act to coordinate cell proliferation within the stem cell niche.

Vegetative shoot identity

Plants go through a series of transitions during their development, switching from embryonic to post-embryonic growth, and then progressing through juvenile and adult stages of vegetative development (a process known as vegetative phase change) before initiating reproductive growth [29,30]. Vegetative phase change and the transition to flowering are regulated throughout the angiosperms by miR156 [31–37] and miR172 [38,39] (Figure 1B). miR156 targets members of the *SQUAMOSA PROMOTER BINDING PROTEIN* (*SBP/SPL*) family of transcription factors, which promote the acquisition of adult features [40–45], and mediate the effects of environmental stresses on shoot development [46–49*]. *SPL* expression is repressed early in development by high levels of miR156; as the level of miR156 declines during shoot development, the expression of *SPL* genes increase, resulting in vegetative phase change. In *Arabidopsis*, miR156 directly targets 11 *SPL* genes. The specific functions of many of these genes are not well understood, but loss-of-function mutations in *SPL9* and *SPL15* suggest that these two loci have important roles in vegetative phase change [50,51]. The transcriptional targets of *SPL9* include the flowering time genes *miR172B* [51] and *SUPPRESSOR OF OVEREXPRESSION OF CO1* [43], and *TRICHOMELESS1* and *TRIPTYCHON* [40], both of which repress trichome development in the inflorescence stem. In addition, a considerable degree of *SPL9* activity is dependent on protein-protein interactions with a diverse range of other proteins [45,52**–55].

The genetic network downstream of miR156 has received considerable attention, but less is known about the factors that control miR156 expression. miR156 levels increase, and phase change is delayed, in response to the ablation of leaf primordia in *Arabidopsis*, tobacco and maize [56]. In *Arabidopsis*, this phenomenon is predominantly attributable to an increase in the expression of *MIR156A* and *MIR156C*, and is suppressed by the application of exogenous sugar, which results in transcriptional and post-transcriptional silencing of miR156 [57*,58*]. It appears, therefore, that a mobile signal from pre-existing leaves contributes to the phase status of subsequent leaves. A miR156/*SPL* module-associated non-autonomous signal derived from leaves has also been suggested to determine the plastochron of new leaf primordia [59].

The molecular mechanism for these effects on miR156 function is still unknown. The transcription factors FUSCA3 (FUS3) [60], and AGAMOUS-LIKE 15 (AGL15) and AGL18 [61], have been shown to promote *MIR156A* and *MIR156C* transcription, but it is unclear if these genes play a role in the down-regulation of these miRNAs during vegetative phase change; indeed, this is unlikely in the case of *FUS3* because expression of the gene strongly declines following germination [62]. Mutations in two components of the Cyclin Dependent Kinase 8 (CDK8) module of the Mediator complex, GRAND CENTRAL (GCT) and CENTER CITY (CCT), produce an increase in miR156 [63*]. Because this module acts primarily to repress gene expression, this result suggests that the decline in miR156 levels during vegetative development may be due to the active repression of *MIR156* genes, rather than the loss of a positive regulator of these genes. This conclusion is supported by evidence that loss-of-function mutations in components of PRC1 (*atbmi1a/atbmi1b*) and PRC2 (*curly leaf/swinger*) exhibit a marked increase in *MIR156A* and *MIR156C* expression [64]. PRC1 and PRC2 promote trimethylation of H3K27, a repressive chromosome mark. The effect of these mutations on *MIR156A* and *MIR156C* expression is therefore consistent with the idea that these genes are actively repressed during vegetative phase change, and further suggests that changes in chromatin structure may contribute to their down-regulation. Finally, it should be noted that the miR172 target, AP2, binds near the transcriptional start sites of both *AGL15* and *MIR156E*, suggesting genes downstream of miR156 may feedback to regulate its expression [51,65].

Control of leaf maturation

Early patterning

Leaves are polarized along both their proximal-distal and adaxial/abaxial (dorsal/ventral) axes [66]. Adaxial/abaxial patterning is established by two different classes of sRNAs, ta-siRNAs and miRNAs, which establish spatially distinct adaxial and abaxial domains within the leaf primordium as it emerges from the SAM [67–69].

ta-siRNAs are sRNAs generated from a transcript that is initially cleaved by a miRNA. Cleaved transcripts are transformed into double-stranded molecules by RNA-DEPENDENT RNA POLYMERASE 6 (RDR6), and are then processed into 21 nt siRNAs by DCL4 [2,5]. In *Arabidopsis*, ta-siRNAs involved in leaf polarity are derived from *TAS3*, which is initially cleaved by miR390 via an AGO1/AGO7 complex. *TAS3* ta-siRNAs are produced on the adaxial side of young leaves, where they repress the expression of transcription factors that promote abaxial identity, ARF3 and ARF4 [69–71] (Figure 1C). In contrast, miR165/miR166 are expressed on the abaxial side of leaf primordia, where they restrict the expression of adaxial-promoting HD-ZIP III proteins [67,69]. In both *Arabidopsis* [72] and maize [69], mutants deficient in ta-siRNA production ectopically express miR165/166, indicating that there is a repressive interaction between the sRNA networks that control leaf adaxial/abaxial polarity [73].

Among the traits regulated by miR165/166 and their HD-ZIP III targets is the orientation of cell types in vascular tissue. As a rule, xylem cells are found on the adaxial surface of the leaf and towards the center of the stem, whereas phloem cells face the abaxial leaf surface and the periphery of the stem. In *Arabidopsis*, miR165/166-resistant *REV* alleles cause the

adaxialization of vascular bundles [14], whereas expression of *MIR165* in the *REV* domain leads to perturbed vascular cell differentiation [74]. The altered venation patterns in the miRNA biogenesis *hyponastic leaves1* (*hyl1*) mutant are explained by the reduced miR165 and increased *REV* expression observed in this mutant [75–77]. Interestingly, *mir159a/b* double mutants [78] also have a simplified venation pattern and reduced vascular cell number, suggesting that additional miRNA families function in vascular development.

Cellular proliferation

A number of miRNA networks control the rate and duration of cell division during leaf morphogenesis. miR319 targets genes within the *CINCINNATA* (*CIN*) clade of *TEOSINTE BRANCHED1/CYCLOIDEA/PCF* (*TCP*) transcription factors, which promote cellular differentiation and mitotic arrest [79–81] (Figure 1D) and control leaf form across the angiosperms [82–84]. Over-expression of miR319 prolongs the expansion of the leaf blade and enhances leaf curvature [80,81], whereas loss of miR319 regulation and the corresponding increase in *CIN-TCP* expression leads to premature differentiation, reduced cell proliferation and a simplification of leaf shape [85–87].

CIN-TCPs regulate growth by a number of mechanisms. In *Arabidopsis*, they have been shown to reduce sensitivity to cytokinin [88] and promote gibberellin activity [89]. *CIN-TCPs* also promote the expression of miR396 [87,90], miR164 [86,91] and components of the ta-siRNA pathway [86]. miR396 restricts cell proliferation predominantly through repression of the *GROWTH REGULATING FACTOR* (*GRF*) family of transcription factors. Expression of miR396 increases as leaves age, with the balance of miR396 and *GRFs* determining whether cells enter mitosis or remain in interphase [90,92,93]. miR164-regulated *NAC* genes promote the formation of leaf sinuses in *Arabidopsis* [94–97] and leaflets in species with compound leaves [98,99]. *CIN-TCPs* inhibit this process by binding directly to *NAC* proteins, blocking their ability to regulate transcription [52**]. There is evidence that the gradual increase in leaf serration in successive rosette leaves of *Arabidopsis*, as well as the increase in leaflet number in *Caramine hirsuta*, is mediated by the interaction of miR156-regulated *SPL* proteins with *CIN-TCPs*, which prevents these proteins from interacting with *NAC* proteins, thus allowing *NAC* proteins to generate serrations or leaflets [52**].

miRNA-regulation of the auxin response appears to be particularly important in leaf development. An increase in leaf serration is observed in plants with elevated levels of miR160-regulated *ARF* genes [100,101]. Overexpression of miR847, which targets *IAA28*, leads to prolonged cell proliferation [102*], while altered activity of the auxin-receptor regulator, miR393, affects leaf form in *Arabidopsis* and rice [103–105].

MYB33 and *MYB65* reduce cell proliferation in leaves when they are over-expressed in *mir159a/b* double mutants [78]. However, while they produce defective pollen, *myb33 myb65* double mutants do not have an obvious vegetative phenotype. These results, and the observation that *MYB33* and *MYB65* translational reporters are not expressed in leaves, suggest that *MYB33* and *MYB65* are deleterious to vegetative development and that the function of miR159 is to repress their expression during this phase.

Leaf senescence

Many of the miRNA regulatory modules that function in cell proliferation also affect cell senescence (Figure 1D). The increase in miR396 expression that occurs during leaf aging not only serves as a signal to terminate cell proliferation, but also promotes leaf senescence [106]. Additionally, the miR164 target *ORESARA1/NAC2* promotes age-induced cell death [107]. Due to a progressive increase in expression of its repressors ETHYLENE-INSENSITIVE2 (EIN2) and EIN3 during leaf development, miR164 expression decreases in an age-dependent manner, leading to a concomitant rise in *ORE1/NAC2* activity and leaf senescence [108,109*]. The *CIN-TCP* targets of miR319 promote senescence via the jasmonic acid pathway [110,111] while loss-of-function mutants of ta-siARF target *ARF2* have also been shown to delay senescence [112,113].

Concluding remarks

Recent work on the regulation of vegetative growth has continued to provide insights into the enhanced complexity that sRNAs engender in developmental networks. This complexity stems from a number of features of sRNA biology, not least the fundamentally different mode of regulation they provide as compared with protein-mediated genetic networks. In addition, specificity of target interactions, functional specialization of different sRNA family members, differential biogenesis, unique interactions with AGO proteins and the potential for intercellular movement all provide junctures for further elaboration of genetic regulation [6]. While all the aforementioned elements of sRNA-target interactions are not fully understood, two key determinants of sRNA function require particular attention.

Do sRNAs function quantitatively or qualitatively?

A number of different models have been proposed as to how sRNAs repress their targets. These include a qualitative model in which target transcripts/proteins are completely removed from a particular domain, and quantitative models of target repression as a dose-dependent function of a sRNA:target ratio or a threshold response to a specific sRNA level [3]. Evidence for all of these possibilities is present in studies of vegetative development (Table 1 – for simplicity dose-dependent and threshold models are not distinguished). For example, miR159 completely represses *MYB33* and *MYB65* expression during vegetative growth [78] while miR165/miR166 and their *HD-ZIP III* targets accumulate in mutually exclusive domains in leaves [11,67,73]. Conversely, a quantitative relationship between sRNAs and their targets has been suggested, for example, by studies of the repression of *CUC* genes by miR164 [25,94] and *TCP* genes by miR319 [82,85]. An all-or-none response to a sRNA threshold is implicit in the ta-siARF concentration gradient model of ARF3 accumulation [70]. Clearly, there is no overarching model for sRNA regulation and, indeed, regulatory mechanisms can be tissue specific [114,115] and dependent on target complementarity [116**]. While it is technically relatively simple to determine whether sRNAs qualitatively ‘clear’ target genes from accumulating in particular domains, current experimental approaches make it difficult to distinguish between dose-dependent and threshold models of regulation. Constitutive overexpression of sRNAs provides no information as to any potential quantitative mechanism and can lead to misleading results (for example the role or otherwise of miR164 in embryonic SAM formation described

earlier). Loss of sRNA function mutants and sRNA resistant alleles provide more robust evidence as to the endogenous role of sRNAs but also make it difficult to draw conclusions about functional modes. Instead, accurate quantification of sRNA and target accumulation in either allelic series of sRNA mutants or through subtly altering sRNA expression will provide clues as to dose-dependent or threshold responses. In cases where sRNA expression changes over developmental time or in different tissues, such subtle expression gradients already exist.

How pervasive is translational repression?

Another key issue that the current literature does not fully address is the extent to which miRNA activity is achieved through translational repression. The pervasive, and likely correct, view in the field is that plant miRNAs predominantly function through target transcript cleavage [1,2]. Indeed, even in reported occurrences of translational repression, the target miRNA sites are also subject to cleavage [39,78,117,118]. In addition, despite recent advances as to the molecular components [119] and intracellular location [120] of translational repression, the mechanism remains unknown. Transcript cleavage by miRNAs is relatively trivial to determine experimentally while the quantification of repression at the protein level requires specific antibodies or translational fusions to measurable reporter peptides. Although the latter approaches are more demanding, they are necessary to determine the full scope of the effects of miRNAs on plant development. This is especially the case in instances where miRNAs seem to differentially affect the expression of targets in multi-gene families – a lack of change at the RNA-level may not reflect an absence of miRNA regulation.

Acknowledgements

We thank members of the Poethig lab for insightful discussions and Sayuri Ando for figure artwork. Work on sRNAs in the lab is funded by NIH grant GM51893.

References and recommended reading

1. Rogers K, Chen X. Biogenesis, turnover, and mode of action of plant microRNAs. *Plant Cell*. 2013; 25:2383–2399. [PubMed: 23881412]
2. Bologna NG, Voinnet O. The diversity, biogenesis, and activities of endogenous silencing small RNAs in Arabidopsis. *Annu Rev Plant Biol*. 2014; 65:473–503. [PubMed: 24579988]
3. Benkovic AH, Timmermans MC. Developmental patterning by gradients of mobile small RNAs. *Curr Opin Genet Dev*. 2014; 27:83–91. [PubMed: 24929831]
4. Rubio-Somoza I, Weigel D. MicroRNA networks and developmental plasticity in plants. *Trends Plant Sci*. 2011; 16:258–264. [PubMed: 21466971]
5. Axtell MJ. Classification and comparison of small RNAs from plants. *Annu Rev Plant Biol*. 2013; 64:137–159. [PubMed: 23330790]
6. Li J, Reichel M, Li Y, Millar AA. The functional scope of plant microRNA-mediated silencing. *Trends Plant Sci*. 2014; 19:750–756. [PubMed: 25242049]
7. Hisanaga T, Miyashima S, Nakajima K. Small RNAs as positional signal for pattern formation. *Curr Opin Plant Biol*. 2014; 21:37–42. [PubMed: 25005923]
8. Barton MK. Twenty years on: The inner workings of the shoot apical meristem, a developmental dynamo. *Dev Biol*. 2010; 341:95–113. [PubMed: 19961843]

9. Barton MK, Poethig RS. Formation of the shoot apical meristem in *Arabidopsis thaliana*: an analysis of development in the wild type and in the shoot meristemless mutant. *Development*. 1993; 119:823–831.
10. McConnell JR, Barton MK. Leaf polarity and meristem formation in *Arabidopsis*. *Development*. 1998; 125:2935–2942. [PubMed: 9655815]
11. 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]
12. Mallory AC, Reinhart BJ, Jones-Rhoades MW, Tang GL, Zamore PD, Barton MK, Bartel DP. MicroRNA control of PHABULOSA in leaf development: importance of pairing to the microRNA 5' region. *EMBO Journal*. 2004; 23:3356–3364. [PubMed: 15282547]
13. Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP. Prediction of plant microRNA targets. *Cell*. 2002; 110:513–520. [PubMed: 12202040]
14. 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]
15. 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]
16. 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.
17. 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. [PubMed: 21483759]
18. 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. [PubMed: 19054365]
19. 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. [PubMed: 21496644]
20. Zhou Y, Honda M, Zhu H, Zhang Z, Guo X, Li T, Li Z, Peng X, Nakajima K, Duan L, et al. Spatiotemporal sequestration of miR165/166 by *Arabidopsis* ARGONAUTE10 promotes shoot apical meristem maintenance. *Cell Rep*. 2015; 10:1819–1827. [PubMed: 25801022]
21. Aida M, Ishida T, Tasaka M. Shoot apical meristem and cotyledon formation during *Arabidopsis* embryogenesis: interaction among the CUP-SHAPED COTYLEDON and SHOOTMERISTEMLESS genes. *Development*. 1999; 126:1563–1570. [PubMed: 10079219]
22. Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M. Genes involved in organ separation in *Arabidopsis*: An analysis of the cup-shaped cotyledon mutant. *Plant Cell*. 1997; 9:841–857. [PubMed: 9212461]
23. Laufs P, Peaucelle A, Morin H, Traas J. MicroRNA regulation of the CUC genes is required for boundary size control in *Arabidopsis* meristems. *Development*. 2004; 131:4311–4322. [PubMed: 15294871]
24. Mallory AC, Dugas DV, Bartel DP, Bartel B. MicroRNA regulation of NAC-domain targets is required for proper formation and separation of adjacent embryonic, vegetative, and floral organs. *Curr Biol*. 2004; 14:1035–1046. [PubMed: 15202996]
25. Sieber P, Wellmer F, Gheyselinck J, Riechmann JL, Meyerowitz EM. Redundancy and specialization among plant microRNAs: role of the MIR164 family in developmental robustness. *Development*. 2007; 134:1051–1060. [PubMed: 17287247]
- 26**. Knauer S, Holt AL, Rubio-Somoza I, Tucker EJ, Hinze A, Pisch M, Javelle M, Timmermans MC, Tucker MR, Laux T. A protodermal miR394 signal defines a region of stem cell competence in the *Arabidopsis* shoot meristem. *Dev Cell*. 2013; 24:125–132. [PubMed: 23333352] The authors identify miR394 as a novel regulator of stem cell maintenance using a sensitized mutant screen. miR394 acts non-cell autonomously from its site of production in the L1 cell layer to potentiate underlying cells to WUS signalling.

27. Schoof H, Lenhard M, Haecker A, Mayer KFX, Jurgens 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. [PubMed: 10761929]
28. 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. [PubMed: 25363783]
29. Huijser P, Schmid M. The control of developmental phase transitions in plants. *Development*. 2011; 138:4117–4129. [PubMed: 21896627]
30. Poethig RS. Vegetative phase change and shoot maturation in plants. *Curr Top Dev Biol*. 2013; 105:125–152. [PubMed: 23962841]
31. Wang JW, Park MY, Wang LJ, Koo Y, Chen XY, Weigel D, Poethig RS. miRNA control of vegetative phase change in trees. *PLoS Genet*. 2011; 7:e1002012. [PubMed: 21383862]
32. Wang Y, Wu F, Bai J, He Y. BrpSPL9 (Brassica rapa ssp. pekinensis SPL9) controls the earliness of heading time in Chinese cabbage. *Plant Biotechnol J*. 2014; 12:312–321. [PubMed: 24237584]
33. Zhang T, Wang J, Zhou C. The role of miR156 in developmental transitions in *Nicotiana tabacum*. *Sci China Life Sci*. 2015; 58:253–260. [PubMed: 25682394]
34. Zhang X, Zou Z, Zhang J, Zhang Y, Han Q, Hu T, Xu X, Liu H, Li H, Ye Z. Over-expression of sly-miR156a in tomato results in multiple vegetative and reproductive trait alterations and partial phenocopy of the sft mutant. *FEBS Lett*. 2011; 585:435–439. [PubMed: 21187095]
35. Chuck G, Cigan AM, Saeteurn K, Hake S. The heterochronic maize mutant *Corngrass1* results from overexpression of a tandem microRNA. *Nat Genet*. 2007; 39:544–549. [PubMed: 17369828]
36. Xie KB, Wu CQ, Xiong LZ. Genomic organization, differential expression, and interaction of SQUAMOSA promoter-binding-like transcription factors and microRNA156 in rice. *Plant Physiol*. 2006; 142:280–293. [PubMed: 16861571]
37. Schwab R, Palatnik JF, Riester M, Schommer C, Schmid M, Weigel D. Specific effects of microRNAs on the plant transcriptome. *Dev Cell*. 2005; 8:517–527. [PubMed: 15809034]
38. Aukerman MJ, Sakai H. Regulation of flowering time and floral organ identity by a microRNA and its APETALA2-like target genes. *Plant Cell*. 2003; 15:2730–2741. [PubMed: 14555699]
39. Chen XM. A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. *Science*. 2004; 303:2022–2025. [PubMed: 12893888]
40. Yu N, Cai WJ, Wang S, Shan CM, Wang LJ, Chen XY. Temporal control of trichome distribution by microRNA156-targeted SPL genes in Arabidopsis thaliana. *Plant Cell*. 2010; 22:2322–2335. [PubMed: 20622149]
41. Shikata M, Koyama T, Mitsuda N, Ohme-Takagi M. Arabidopsis SBP-box genes SPL10, SPL11 and SPL2 control morphological change in association with shoot maturation in the reproductive phase. *Plant Cell Physiol*. 2009; 50:2133–2145. [PubMed: 19880401]
42. Yamaguchi A, Wu MF, Yang L, Wu G, Poethig RS, Wagner D. The microRNA-regulated SBP-Box transcription factor SPL3 is a direct upstream activator of LEAFY, FRUITFULL, and APETALA1. *Dev Cell*. 2009; 17:268–278. [PubMed: 19686687]
43. Wang JW, Czech B, Weigel D. miR156-regulated SPL transcription factors define an endogenous flowering pathway in Arabidopsis thaliana. *Cell*. 2009; 138:738–749. [PubMed: 19703399]
44. Yu ZX, Wang LJ, Zhao B, Shan CM, Zhang YH, Chen DF, Chen XY. Progressive regulation of sesquiterpene biosynthesis in Arabidopsis and Patchouli (*Pogostemon cablin*) by the miR156-targeted SPL transcription factors. *Mol Plant*. 2015; 8:98–110. [PubMed: 25578275]
45. Gou JY, Felippes FF, Liu CJ, Weigel D, Wang JW. Negative regulation of anthocyanin biosynthesis in Arabidopsis by a miR156-targeted SPL transcription factor. *Plant Cell*. 2011; 23:1512–1522. [PubMed: 21487097]
46. Stief A, Altmann S, Hoffmann K, Pant BD, Scheible WR, Baurle I. Arabidopsis miR156 regulates tolerance to recurring environmental stress through SPL transcription factors. *Plant Cell*. 2014; 26:1792–1807. [PubMed: 24769482]
47. Cui LG, Shan JX, Shi M, Gao JP, Lin HX. The miR156-SPL9-DFR pathway coordinates the relationship between development and abiotic stress tolerance in plants. *Plant J*. 2014; 80:1108–1117. [PubMed: 25345491]

- 48*. Bergonzi S, Albani MC, Ver Loren van Themaat E, Nordstrom KJ, Wang R, Schneeberger K, Moerland PD, Coupland G. Mechanisms of age-dependent response to winter temperature in perennial flowering of *Arabis alpina*. *Science*. 2013; 340:1094–1097. [PubMed: 23723236] In tandem with [49], this study demonstrates an age-dependent repression of flowering in perennial plants mediated by miR156. High miR156 expression maintains the vegetative phase until inductive environmental conditions permit a transition to flowering.
- 49*. Zhou CM, Zhang TQ, Wang X, Yu S, Lian H, Tang H, Feng ZY, Zozomova-Lihova J, Wang JW. Molecular basis of age-dependent vernalization in *Cardamine flexuosa*. *Science*. 2013; 340:1097–1100. [PubMed: 23723237] See [48]
50. Schwarz S, Grande AV, Bujdoso N, Saedler H, Huijser P. The microRNA regulated SBP-box genes SPL9 and SPL15 control shoot maturation in *Arabidopsis*. *Plant Mol Biol*. 2008; 67:183–195. [PubMed: 18278578]
51. Wu G, Park MY, Conway SR, Wang JW, Weigel D, Poethig RS. The sequential action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. *Cell*. 2009; 138:750–759. [PubMed: 19703400]
- 52*. Rubio-Somoza I, Zhou CM, Confraria A, Martinho C, von Born P, Baena-Gonzalez E, Wang JW, Weigel D. Temporal control of leaf complexity by miRNA-regulated licensing of protein complexes. *Curr Biol*. 2014; 24:2714–2719. [PubMed: 25448000] This study describes the convergence of three miRNA pathways in the regulation of age-dependent increases in leaf complexity. Altered expression of these miRNAs during development allows distinct post-translational interactions between the miRNA targets to modify leaf form.
53. Zhang TQ, Lian H, Tang H, Dolezal K, Zhou CM, Yu S, Chen JH, Chen Q, Liu H, Ljung K, et al. An intrinsic microRNA timer regulates progressive decline in shoot regenerative capacity in plants. *Plant Cell*. 2015; 27:349–360. [PubMed: 25649435]
54. Yu S, Galvao VC, Zhang YC, Horrer D, Zhang TQ, Hao YH, Feng YQ, Wang S, Schmid M, Wang JW. Gibberellin regulates the *Arabidopsis* floral transition through miR156-targeted SQUAMOSA promoter binding-like transcription factors. *Plant Cell*. 2012; 24:3320–3332. [PubMed: 22942378]
55. Xue XY, Zhao B, Chao LM, Chen DY, Cui WR, Mao YB, Wang LJ, Chen XY. Interaction between two timing microRNAs controls trichome distribution in *Arabidopsis*. *PLoS Genet*. 2014; 10:e1004266. [PubMed: 24699192]
56. Yang L, Conway SR, Poethig RS. Vegetative phase change is mediated by a leaf-derived signal that represses the transcription of miR156. *Development*. 2011; 138:245–249. [PubMed: 21148189]
- 57*. Yang L, Xu M, Koo Y, He J, Poethig RS. Sugar promotes vegetative phase change in *Arabidopsis thaliana* by repressing the expression of MIR156A and MIR156C. *eLife*. 2013; 2:e00260. [PubMed: 23538384] Together with [58], this study demonstrates a clear role for sugar in the repression of miR156 during plant aging. As such, this provides a framework for how plants might use endogenous signals to determine developmental age.
- 58*. Yu S, Cao L, Zhou CM, Zhang TQ, Lian H, Sun Y, Wu JQ, Huang JR, Wang GD, Wang JW. Sugar is an endogenous cue for juvenile-to-adult phase transition in plants. *eLife*. 2013; 2:e00269. [PubMed: 23543845] See [57]
59. Wang JW, Schwab R, Czech B, Mica E, Weigel D. Dual effects of miR156-targeted SPL genes and CYP78A5/KLUH on plastochron length and organ size in *Arabidopsis thaliana*. *Plant Cell*. 2008; 20:1231–1243. [PubMed: 18492871]
60. Wang F, Perry SE. Identification of direct targets of FUSCA3, a key regulator of *Arabidopsis* seed development. *Plant Physiol*. 2013; 161:1251–1264. [PubMed: 23314941]
61. Serivichyaswat P, Ryu HS, Kim W, Kim S, Chung KS, Kim JJ, Ahn JH. Expression of the floral repressor miRNA156 is positively regulated by the AGAMOUS-like proteins AGL15 and AGL18. *Mol Cells*. 2015; 38:259–266. [PubMed: 25666346]
62. Lumba S, Tsuchiya Y, Delmas F, Hezky J, Provart NJ, Shi Lu Q, McCourt P, Gazzarrini S. The embryonic leaf identity gene FUSCA3 regulates vegetative phase transitions by negatively modulating ethylene-regulated gene expression in *Arabidopsis*. *BMC Biol*. 2012; 10:8. [PubMed: 22348746]

- 63*. Gillmor CS, Silva-Ortega CO, Willmann MR, Buendia-Monreal M, Poethig RS. The Arabidopsis Mediator CDK8 module genes CCT (MED12) and GCT (MED13) are global regulators of developmental phase transitions. *Development*. 2014; 141:4580–4589. [PubMed: 25377553]
Analysis of mutations in Mediator complex genes cct and gct reveal roles for the Mediator complex in the regulation of phase transitions throughout plant development, in addition to the roles previously described during embryogenesis. The Mediator complex acts at least in part through repression of miR156.
64. Pico S, Ortiz-Marchena MI, Merini W, Calonje M. Deciphering the role of Polycomb Repressive Complex 1 (PRC1) variants in regulating the acquisition of flowering competence in Arabidopsis. *Plant Physiol*. 2015
65. Yant L, Mathieu J, Dinh TT, Ott F, Lanz C, Wollmann H, Chen XM, Schmid M. Orchestration of the floral transition and floral development in Arabidopsis by the bifunctional transcription factor APETALA2. *Plant Cell*. 2010; 22:2156–2170. [PubMed: 20675573]
66. Steeves, TA.; Sussex, IM. Patterns in plant development. 2nd. Cambridge England; New York: Cambridge University Press; 1989.
67. Juarez MT, Kui JS, Thomas J, Heller BA, Timmermans MCP. microRNA-mediated repression of rolled leaf1 specifies maize leaf polarity. *Nature*. 2004; 428:84–88. [PubMed: 14999285]
68. Kidner CA, Martienssen RA. Spatially restricted microRNA directs leaf polarity through ARGONAUTE1. *Nature*. 2004; 428:81–84. [PubMed: 14999284]
69. Nogueira FT, Madi S, Chitwood DH, Juarez MT, Timmermans MC. Two small regulatory RNAs establish opposing fates of a developmental axis. *Genes Dev*. 2007; 21:750–755. [PubMed: 17403777]
70. Chitwood DH, Nogueira FT, Howell MD, Montgomery TA, Carrington JC, Timmermans MC. Pattern formation via small RNA mobility. *Genes Dev*. 2009; 23:549–554. [PubMed: 19270155]
71. Schwab R, Maizel A, Ruiz-Ferrer V, Garcia D, Bayer M, Crespi M, Voinnet O, Martienssen RA. Endogenous TasiRNAs mediate non-cell autonomous effects on gene regulation in Arabidopsis thaliana. *PLoS One*. 2009; 4:e5980. [PubMed: 19543387]
72. Li H, Xu L, Wang H, Yuan Z, Cao X, Yang Z, Zhang D, Xu Y, Huang H. The putative RNA-dependent RNA polymerase RDR6 acts synergistically with ASYMMETRIC LEAVES1 and 2 to repress BREVIPEDICELLUS and MicroRNA165/166 in Arabidopsis leaf development. *Plant Cell*. 2005; 17:2157–2171. [PubMed: 16006579]
73. Nogueira FT, Chitwood DH, Madi S, Ohtsu K, Schnable PS, Scanlon MJ, Timmermans MC. Regulation of small RNA accumulation in the maize shoot apex. *PLoS Genet*. 2009; 5:e1000320. [PubMed: 19119413]
74. Ilegems M, Douet V, Meylan-Bettex M, Uyttewaal M, Brand L, Bowman JL, Stieger PA. Interplay of auxin, KANADI and Class III HD-ZIP transcription factors in vascular tissue formation. *Development*. 2010; 137:975–984. [PubMed: 20179097]
75. Yu L, Yu X, Shen R, He Y. HYL1 gene maintains venation and polarity of leaves. *Planta*. 2005; 221:231–242. [PubMed: 15580355]
76. Jover-Gil S, Candela H, Robles P, Aguilera V, Barrero JM, Micol JL, Ponce MR. The microRNA pathway genes AGO1, HEN1 and HYL1 participate in leaf proximal-distal, venation and stomatal patterning in Arabidopsis. *Plant Cell Physiol*. 2012; 53:1322–1333. [PubMed: 22623415]
77. Liu Z, Jia L, Wang H, He Y. HYL1 regulates the balance between adaxial and abaxial identity for leaf flattening via miRNA-mediated pathways. *J Exp Bot*. 2011; 62:4367–4381. [PubMed: 21610018]
78. Alonso-Peral MM, Li J, Li Y, Allen RS, Schnippenkoetter W, Ohms S, White RG, Millar AA. The microRNA159-regulated GAMYB-like genes inhibit growth and promote programmed cell death in Arabidopsis. *Plant Physiol*. 2010; 154:757–771. [PubMed: 20699403]
79. Nath U, Crawford BCW, Carpenter R, Coen E. Genetic control of surface curvature. *Science*. 2003; 299:1404–1407. [PubMed: 12610308]
80. Efroni I, Blum E, Goldshmidt A, Eshed Y. A protracted and dynamic maturation schedule underlies Arabidopsis leaf development. *Plant Cell*. 2008; 20:2293–2306. [PubMed: 18805992]
81. Palatnik JF, Allen E, Wu XL, Schommer C, Schwab R, Carrington JC, Weigel D. Control of leaf morphogenesis by microRNAs. *Nature*. 2003; 425:257–263. [PubMed: 12931144]

82. Shleizer-Burko S, Burko Y, Ben-Herzel O, Ori N. Dynamic growth program regulated by LANCEOLATE enables flexible leaf patterning. *Development*. 2011; 138:695–704. [PubMed: 21228002]
83. Zhou M, Li D, Li Z, Hu Q, Yang C, Zhu L, Luo H. Constitutive expression of a miR319 gene alters plant development and enhances salt and drought tolerance in transgenic creeping bentgrass. *Plant Physiol*. 2013; 161:1375–1391. [PubMed: 23292790]
84. Mao Y, Wu F, Yu X, Bai J, Zhong W, He Y. MicroRNA319a–targeted *Brassica rapa* ssp. *pekinensis* TCP genes modulate head shape in chinese cabbage by differential cell division arrest in leaf regions. *Plant Physiol*. 2014; 164:710–720. [PubMed: 24351684]
85. Ori N, Cohen AR, Etzioni A, Brand A, Yanai O, Shleizer S, Menda N, Amsellem Z, Efroni I, Pekker I, et al. Regulation of LANCEOLATE by miR319 is required for compound-leaf development in tomato. *Nat Genet*. 2007; 39:787–791. [PubMed: 17486095]
86. Koyama T, Mitsuda N, Seki M, Shinozaki K, Ohme-Takagi M. TCP transcription factors regulate the activities of ASYMMETRIC LEAVES1 and miR164, as well as the auxin response, during differentiation of leaves in *Arabidopsis*. *Plant Cell*. 2010; 22:3574–3588. [PubMed: 21119060]
87. Schommer C, Debernardi JM, Bresso EG, Rodriguez RE, Palatnik JF. Repression of cell proliferation by miR319-regulated TCP4. *Mol Plant*. 2014; 7:1533–1544. [PubMed: 25053833]
88. Efroni I, Han SK, Kim HJ, Wu MF, Steiner E, Birnbaum KD, Hong JC, Eshed Y, Wagner D. Regulation of leaf maturation by chromatin-mediated modulation of cytokinin responses. *Developmental Cell*. 2013; 24:438–445. [PubMed: 23449474]
89. Yanai O, Shani E, Russ D, Ori N. Gibberellin partly mediates LANCEOLATE activity in tomato. *Plant J*. 2011; 68:571–582. [PubMed: 21771122]
90. Rodriguez RE, Mecchia MA, Debernardi JM, Schommer C, Weigel D, Palatnik JF. Control of cell proliferation in *Arabidopsis thaliana* by microRNA miR396. *Development*. 2010; 137:103–112. [PubMed: 20023165]
91. Koyama T, Furutani M, Tasaka M, Ohme-Takagi M. TCP transcription factors control the morphology of shoot lateral organs via negative regulation of the expression of boundary-specific genes in *Arabidopsis*. *Plant Cell*. 2007; 19:473–484. [PubMed: 17307931]
92. Liu DM, Song Y, Chen ZX, Yu DQ. Ectopic expression of miR396 suppresses RF target gene expression and alters leaf growth in *Arabidopsis*. *Physiol Plant*. 2009; 136:223–236. [PubMed: 19453503]
93. Wang L, Gu XL, Xu DY, Wang W, Wang H, Zeng MH, Chang ZY, Huang H, Cui XF. miR396-targeted AtGRF transcription factors are required for coordination of cell division and differentiation during leaf development in *Arabidopsis*. *J Exp Bot*. 2011; 62:761–773. [PubMed: 21036927]
94. Nikovics K, Blein T, Peaucelle A, Ishida T, Morin H, Aida M, Laufs P. The balance between the MIR164A and CUC2 genes controls leaf margin serration in *Arabidopsis*. *Plant Cell*. 2006; 18:2929–2945. [PubMed: 17098808]
95. Bilsborough GD, Runions A, Barkoulas M, Jenkins HW, Hasson A, Galinha C, Laufs P, Hay A, Prusinkiewicz P, Tsiantis M. Model for the regulation of *Arabidopsis thaliana* leaf margin development. *Proc Natl Acad Sci USA*. 2011; 108:3424–3429. [PubMed: 21300866]
96. Hasson A, Plessis A, Blein T, Adroher B, Grigg S, Tsiantis M, Boudaoud A, Damerval C, Laufs P. Evolution and diverse roles of the CUP-SHAPED COTYLEDON genes in *Arabidopsis* leaf development. *Plant Cell*. 2011; 23:54–68. [PubMed: 21258003]
97. Larue CT, Wen J, Walker JC. A microRNA-transcription factor module regulates lateral organ size and patterning in *Arabidopsis*. *Plant J*. 2009; 58:450–463. [PubMed: 19154203]
98. Blein T, Pulido A, Vialette-Guiraud A, Nikovics K, Morin H, Hay A, Johansen IE, Tsiantis M, Laufs P. A conserved molecular framework for compound leaf development. *Science*. 2008; 322:1835–1839. [PubMed: 19095941]
99. Berger Y, Harpaz-Saad S, Brand A, Melnik H, Sirding N, Alvarez JP, Zinder M, Samach A, Eshed Y, Ori N. The NAC-domain transcription factor GOBLET specifies leaflet boundaries in compound tomato leaves. *Development*. 2009; 136:823–832. [PubMed: 19176589]

100. Mallory AC, Bartel DP, Bartel B. MicroRNA-directed regulation of Arabidopsis AUXIN RESPONSE FACTOR17 is essential for proper development and modulates expression of early auxin response genes. *Plant Cell*. 2005; 17:1360–1375. [PubMed: 15829600]
101. Liu PP, Montgomery TA, Fahlgren N, Kasschau KD, Nonogaki H, Carrington JC. Repression of AUXIN RESPONSE FACTOR10 by microRNA160 is critical for seed germination and post-germination stages. *Plant J*. 2007; 52:133–146. [PubMed: 17672844]
- 102*. Wang JJ, Guo HS. Cleavage of INDOLE-3-ACETIC ACID INDUCIBLE28 mRNA by microRNA847 upregulates auxin signaling to modulate cell proliferation and lateral organ growth in Arabidopsis. *Plant Cell*. 2015; 27:574–590. [PubMed: 25794935] The authors demonstrate a novel role for miR847 in auxin signalling through cleavage of the the Aux/IAA repressor IAA28
103. Si-Ammour A, Windels D, Arn-Boulidoires E, Kutter C, Ailhas J, Meins F Jr, Vazquez F. miR393 and secondary siRNAs regulate expression of the TIR1/AFB2 auxin receptor clade and auxin-related development of Arabidopsis leaves. *Plant Physiol*. 2011; 157:683–691. [PubMed: 21828251]
104. Xia K, Wang R, Ou X, Fang Z, Tian C, Duan J, Wang Y, Zhang M. OsTIR1 and OsAFB2 downregulation via OsmiR393 overexpression leads to more tillers, early flowering and less tolerance to salt and drought in rice. *PLoS One*. 2012; 7:e30039. [PubMed: 22253868]
105. Bian H, Xie Y, Guo F, Han N, Ma S, Zeng Z, Wang J, Yang Y, Zhu M. Distinctive expression patterns and roles of the miRNA393/TIR1 homolog module in regulating flag leaf inclination and primary and crown root growth in rice (*Oryza sativa*). *New Phytol*. 2012; 196:149–161. [PubMed: 22846038]
106. Debernardi JM, Mecchia MA, Vercruyssen L, Smaczniak C, Kaufmann K, Inze D, Rodriguez RE, Palatnik JF. Post-transcriptional control of GRF transcription factors by microRNA miR396 and GIF co-activator affects leaf size and longevity. *Plant J*. 2014; 79:413–426. [PubMed: 24888433]
107. Oh SA, Park JH, Lee GI, Paek KH, Park SK, Nam HG. Identification of three genetic loci controlling leaf senescence in Arabidopsis thaliana. *Plant J*. 1997; 12:527–535. [PubMed: 9351240]
108. Kim JH, Woo HR, Kim J, Lim PO, Lee IC, Choi SH, Hwang D, Nam HG. Trifurcate Feed-Forward Regulation of Age-Dependent Cell Death Involving miR164 in Arabidopsis. *Science*. 2009; 323:1053–1057. [PubMed: 19229035]
- 109*. Li Z, Peng J, Wen X, Guo H. ETHYLENE-INSENSITIVE3 is a senescence-associated gene that accelerates age-dependent leaf senescence by directly repressing miR164 transcription in Arabidopsis. *Plant Cell*. 2013; 25:3311–3328. [PubMed: 24064769] This study further elucidates the mechanism of age-dependent leaf senescence through showing direct repression of miR164 by EIN3. Expression levels of EIN3 increase during leaf aging, leading to a reduction in miR164 and a concomitant increase in expression of the senescence promoting miR164-target NAC2
110. Schommer C, Palatnik JF, Aggarwal P, Chetelat A, Cubas P, Farmer EE, Nath U, Weigel D. Control of jasmonate biosynthesis and senescence by miR319 targets. *PLoS Biol*. 2008; 6:e230. [PubMed: 18816164]
111. Sarvepalli K, Nath U. Hyper-activation of the TCP4 transcription factor in Arabidopsis thaliana accelerates multiple aspects of plant maturation. *Plant J*. 2011; 67:595–607. [PubMed: 21518050]
112. Ellis CM, Nagpal P, Young JC, Hagen G, Guilfoyle TJ, Reed JW. AUXIN RESPONSE FACTOR1 and AUXIN RESPONSE FACTOR2 regulate senescence and floral organ abscission in Arabidopsis thaliana. *Development*. 2005; 132:4563–4574. [PubMed: 16176952]
113. Lim PO, Lee IC, Kim J, Kim HJ, Ryu JS, Woo HR, Nam HG. AUXIN RESPONSE FACTOR2 (ARF2) plays a major role in regulating auxin-mediated leaf longevity. *J Exp Bot*. 2010; 61:1419–1430. [PubMed: 20164142]
114. Alonso-Peral MM, Sun C, Millar AA. MicroRNA159 can act as a switch or tuning microRNA independently of its abundance in Arabidopsis. *PLoS One*. 2012; 7:e34751. [PubMed: 22511963]
115. 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]

- 116**. Li J, Reichel M, Millar AA. Determinants beyond both complementarity and cleavage govern microR159 efficacy in Arabidopsis. *PLoS Genet.* 2014; 10:e1004232. [PubMed: 24626050] By generating mismatches between miR159 and its targets MYB33/MYB65, the authors demonstrate that complete miRNA:target complementarity is not essential for efficient silencing. Furthermore, they show that miRNA:target stoichiometry and broader sequence context are also important factors in silencing efficiency.
117. 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]
118. Gandikota M, Birkenbihl RP, Hohmann S, Cardon GH, Saedler H, Huijser P. The miRNA156/157 recognition element in the 3' UTR of the Arabidopsis SBP box gene SPL3 prevents early flowering by translational inhibition in seedlings. *Plant J.* 2007; 49:683–693. [PubMed: 17217458]
119. Yang L, Wu G, Poethig RS. Mutations in the GW-repeat protein SUO reveal a developmental function for microRNA-mediated translational repression in Arabidopsis. *Proc Natl Acad Sci USA.* 2012; 109:315–320. [PubMed: 22184231]
120. Li S, Liu L, Zhuang X, Yu Y, Liu X, Cui X, Ji L, Pan Z, Cao X, Mo B, et al. MicroRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in Arabidopsis. *Cell.* 2013; 153:562–574. [PubMed: 23622241]
121. Wollmann H, Mica E, Todesco M, Long JA, Weigel D. On reconciling the interactions between APETALA2, miR172 and AGAMOUS with the ABC model of flower development. *Development.* 2010; 137:3633–3642. [PubMed: 20876650]
122. Allen RS, Li J, Alonso-Peral MM, White RG, Gubler F, Millar AA. MicroR159 regulation of most conserved targets in Arabidopsis has negligible phenotypic effects. *Silence.* 2010; 1:18. [PubMed: 21029441]

Highlights

- sRNA networks are key regulators of diverse aspects of vegetative growth
- Increasing evidence suggests multiple developmental roles for certain sRNAs
- The extent of quantitative regulation and translational repression are key unknowns

Figure 1.

Models of sRNA activity during vegetative shoot development. A) miRNA activity during SAM specification (adapted from [20,26]). B) The role of sRNAs in vegetative phase change (note it is unclear where in the shoot apex juvenile identity is specified). C) Opposing gradients of sRNA accumulation regulate adaxial/abaxial patterning in the leaf. D) Numerous miRNA networks have been shown to affect the balance between cell proliferation and senescence during leaf development. Arrows indicate positive genetic relationship, T-bars indicate repressive genetic relationships. In B) dotted line depicts

inhibition of miR165/166 movement by AGO10, in C) and D) dotted line indicates speculative interactions.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Functional summary of the best characterized sRNA regulators of vegetative shoot growth.

Table 1

sRNA	Vegetative function	Target genes	Evidence for translational repression	Mechanism of repression in vegetative shoot tissue (other tissue types)	References
miR156	Repress vegetative phase change Plastochron regulation	<i>SPL</i> family	Yes	Quantitative	[51,59,118,119]
miR172	Promote adult leaf morphology	<i>AP2</i> family	Yes	Quantitative	[39,121]
miR165/ miR166	Limit adaxial leaf development Restrict meristematic growth	<i>HD-ZIP III</i>	No	Qualitative (quantitative in root)	[73,115]
miR164	Regulate leaf serration/comple xity Delay senescence	<i>NAC</i> family	No	Quantitative	[25,94]
miR394	Embryonic SAM maintenance	<i>LCR</i>	No	Quantitative	[26]
miR396	Limit cell proliferation Promote senescence	<i>GRF</i> family	No	Quantitative	[90]
miR319	Promote cell proliferation Delay senescence	<i>TCP</i> family	No	Quantitative	[82,85]
miR159	Limit cell proliferation	<i>MYB</i> family	Yes	Qualitative (quantitative during germination)	[78,114,122]
ta-siARF	Limit abaxial leaf development Delay senescence Repress adult leaf traits	<i>ARF2/3/4</i>	No	Quantitative	[70]