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Review

Evolutionary conservation of microRNA regulatory programs in plant flower development

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ABSTRACT

MicroRNAs (miRNAs) are post-transcriptional regulators of growth and development in both plants and animals. Flowering is critical for the reproduction of angiosperms. Flower development entails the transition from vegetative growth to reproductive growth, floral organ initiation, and the development of floral organs. These developmental processes are genetically regulated by miRNAs, which participate in complex genetic networks of flower development. A survey of the literature shows that miRNAs, their specific targets, and the regulatory programs in which they participate are conserved throughout the plant kingdom. This review summarizes the role of miRNAs and their targets in the regulation of gene expression during the floral developmental phase, which includes the floral transition stage, followed by floral patterning, and then the development of floral organs. The conservation patterns observed in each component of the miRNA regulatory system suggest that these miRNAs play important roles in the evolution of flower development.

Introduction

It is normally agreed that plant development can be divided into vegetative and reproductive growth phases. Flowering is critical for the reproduction of angiosperms. During flowering, plants acquire reproductive competence, and can be induced by environmental cues to form floral organs. Flower development can be divided into three developmental phases: the floral transition phase, the floral patterning phase, and the phase of development of floral organs. The floral transition is the transition from the vegetative phase to the reproductive phase. During this transition, the vegetative shoot apical meristem (SAM) or lateral meristem (LM) is converted to floral meristems (FMs) (Liu et al., 2009a, 2009b; Barton, 2010). After completion of the floral transition, FMs give rise to different floral organ primordia during the floral patterning phase. Finally, the floral organ primordia develop in successive whorls, and then grow and differentiate into floral organs.

Recently, significant progress has been made towards understanding the molecular mechanisms that control flowering. Flowering processes are regulated by a very complex set of pathways controlled by numerous genes. These included floral meristem identity genes, floral organ identity genes, and some evolutionary conserved miRNAs (Amasino, 2010; Irish, 2010; Nag and Jack, 2010; Posé et al., 2012; Srikanth and Schmid, 2011). MiRNAs are small (20–25 nucleotides), endogenous, non-coding, single-stranded RNAs, which are found in many organisms, where they regulate gene expression at the post-transcriptional level (Bartel, 2004). MiRNAs negatively regulate certain genes involved in flower development by directing RNA cleavage or inhibiting translation of the target transcripts (Jones-Rhoades et al., 2006). MiRNAs are not only regulators of gene expression patterns, but also play an essential role in the coordination of complex developmental processes through their extensive integration within genetic networks (Huijser and Schmid, 2011; Nag and Jack, 2010; Srikanth and Schmid, 2011; Sun, 2012; Yamaguchi and Abe, 2012; Zhu and Helliwell, 2011).

This review first discusses the conservation of miRNAs and their targets. We then summarize their contribution to three developmental phases of flower development, including the floral transition, the floral patterning, and the development of floral organs. Some miRNAs regulate members of the florigen and integrators involved in flowering, and thus participate in complex genetic networks at floral transition phase. Other miRNAs target and restrict the action of various genes that control different flower-related processes. We also discuss the evolutionary

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conservation of both of these miRNAs and the regulatory pathways in which they participate in various plant species.

Phylogenetic distribution and conservation of nine miRNAs

Phylogenetic distribution

At least nine conserved miRNA families have been reported to play key roles during flower development in plants. These include miR156, miR159, miR160, miR164, miR166/165, miR 167, miR169, miR172, and miR319 (Fig. 1). These miRNAs regulate flower development by targeting various transcription factors involved in flower developmental processes. For example, miR172 regulates floral organ identity and flowering time by translational repression or target cleavage of members of the *APETELA2* (*AP2*) transcription factor genes (Aukerman and Sakai, 2003; Chen, 2004; Glazińska et al., 2009; Jung et al., 2007; Schwab et al., 2005; Varkonyi-Gasic et al., 2012). MiR156 targets *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) transcription factor gene family to control the transition from the vegetative phase to the floral phase in *Arabidopsis*, rice, and maize (Chuck et al., 2007a; Gandikota et al., 2007; Jiao et al., 2010; Miura et al., 2010; Yang et al., 2010). MiR159 is required for normal anther development, which it controls through regulating the expression of genes that encode MYB transcription factors (Achard et al., 2004; Tsuji et al., 2006). Some rice miRNAs are more abundant in pollen than in leaves (Wei et al., 2011). MiR156, miR164, miR166, miR172, and miR167 display marked differences in their levels expressed during the development of female flowers of *Carya cathayensis* (Wang et al., 2012). Most studies about the roles of miRNAs in flowering were conducted using the model plant *Arabidopsis*, although results obtained from maize, rice, tomato, potato, and other non-model plants have indicated a high level of conservation of these miRNAs and the regulatory interactions with their targets across the plant kingdom (Axtell and Bartel, 2005; Willmann and Poethig, 2007; Sunkar and Jagadeeswaran, 2008; Zhang et al., 2006).

Based on the recently released miRBase (Release 19.0: August 2012, Kozomara and Griffiths-Jones, 2011) and some recently published reports, at least 1505 miRNA sequences have been identified, which belong to nine miRNA families (Supplementary Table S1). These 1505 miRNAs were obtained from 58 plant species, which range from angiosperms to bryophytes and include 10 monocotyledonous species, 41 dicotyledonous species, 4 gymnosperms, 2 pteridophytes, and one bryophyte. These 58 species represents 23 families, including some families that occupy key phylogenetic positions, such as Ranunculaceae (basal eudicots), Magnoliaceae (basal angiosperms), Pinaceae (gymnosperms), and Funariacee (mosses). The phylogenetic distribution of the nine miRNA families across various species from all major lineages of land plants (Fig. 2) indicates the high levels of their conservation. Of these miRNA families, miR164 is found in both angiosperms and gymnosperms, and thus appears to be conserved across all spermatophytes. Members of the miR159, miR169, and miR172 families are found in angiosperms, gymnosperms, and ferns, and thus appear to be conserved across all tracheophytes. However, the five remaining families (miR156, miR160, miR166/165, miR167, and miR319) are found not only in dicots and monocots but also in gymnosperms, lycopods, and mosses, and thus appear to be conserved across all embryophytes.

Sequence conservation

The alignments and sequence similarities of these mature miRNAs from various species are shown in the supplementary figure. The sequence alignment of mature miRNAs reveals an overall well-conserved consensus with a few variations. For example, whereas members of the miR164 family are completely identical through 28 species, the mature sequences of miR172 from 37 angiosperm species and miR166 from 36 species have 90% similarity. Mature sequences of miR159, miR160, miR167, miR169, and miR319 are more than 80% similar. Mature miR156 sequences from 40 species are 75% similar (Supplementary Fig.). Members of the miR156 family from gymnosperms display greater sequence diversity than those from angiosperms. However, moss miR156 is

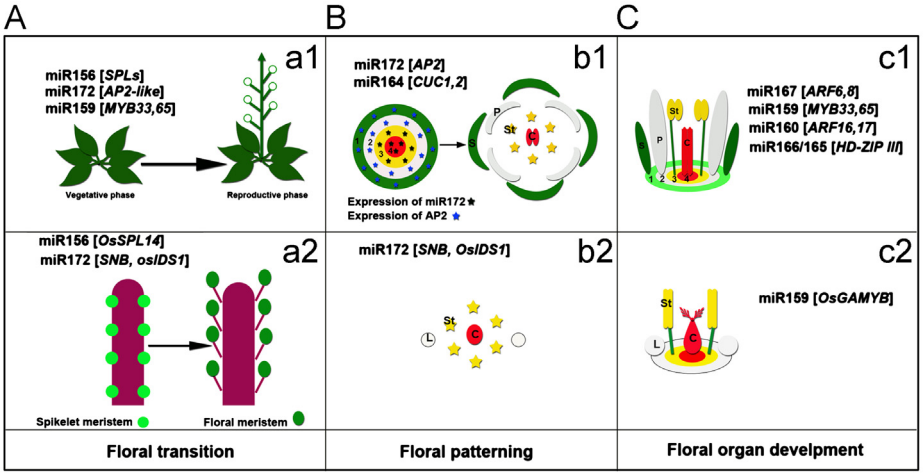

Fig. 1. The flower development in *Arabidopsis* ((a1), (b1) and (c1)) and rice ((a2), (b2), (c2)). The involvement of miRNAs and miRNA-target genes at various phases is indicated. (A) Floral transition: (a1) In *Arabidopsis*, three sets of miRNA-mediated module, miR156-*SPLs*, miR172-*AP2-like*, miR159-*MYBs*, affect floral meristems formation. (a2) In rice, miR156-*OsSPL14* and miR172-*SNB/OsIDS1* modules are involved in spikelet meristem converting into floral meristem. (B) Floral patterning: (b1). In *Arabidopsis*, miR172 functions to restrict *AP2* in the center of the flower, which expresses in whorls 3 and 4 (indicating by black stars). *AP2* transcripts are present in whorls 1 and 2 (indicating by blue stars) to form sepal and petal primordia. miR164 negatively regulates *CUC1*, *CUC2* expression to establish boundaries between and within whorls of organs. (b2) In rice, miR172 and its targets *SNB* and *OsIDS1* participate in regulating lodicules development. (C) Floral organs development: (c1). In *Arabidopsis*, miR159 functions to negatively regulate *MYB33* and *MYB65* in stamens, miR166/165 functions to negatively regulate the *HD-ZIP III* genes in various floral organs, miR167 targets *ARF6* and *ARF8* in stamens, miR160 targets *ARF16* and *ARF17* in reproductive organs, and miR319 targets *TCP* in the petal. (c2) In rice, miR159 targets *OsGAMYB* in stamens. Abbreviation used in this figure: *SPL*, *SQUAMOSA PROMOTER BINGDING PROTEIN-LIKE*; *AP2*, *APETALA2*; *CUC*, *CUP-SHAPED COTYLEDON*; *ARF*, *AUXIN RESPONSE FACTOR*; *HD-ZIP III*, *class III HOMEODOMAINLEUCINE ZIPPER*; *SNB*, *SUPERNUMARY BRACT*; *IDS1*, *INDETERMINATE SPIKELET1*; L, Lodicule; S, Sepal; P, Petal; St, Stamen; C, Carpel.

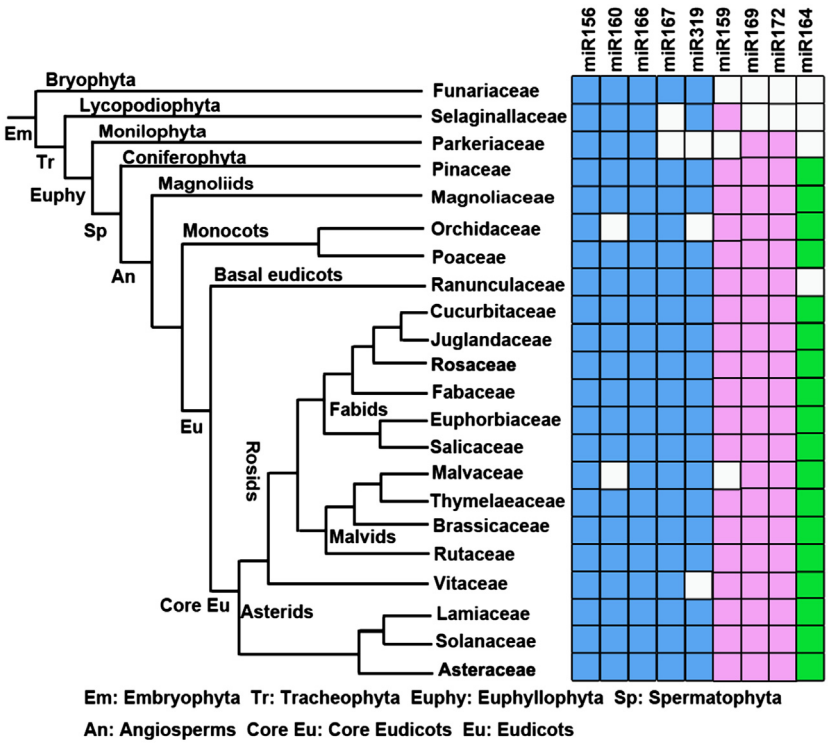

Fig. 2. The phylogenetic distribution of miRNAs involved in flower development in plant kingdom. The presence of miRNAs is indicated by colour boxes, blue ones as embyophytes conserved, pink ones as tracheophytes conserved, green ones as angiosperms conserved. The absence of miRNA is indicated by white ones.

almost identical to miR156 of the most angiosperms, with a different nucleotide found at only a single position.

Conservation of targets

The observation that the target genes of these nine miRNA families are also conserved across different plant families indicates that these miRNAs are conserved in function as well as in sequence. The targets of the nine miRNA families all encode transcription factors (Table 1), which are known to affect flowering. Although there are many nucleotide changes among the targets of different plant species, the sequences of complementary sites are highly conserved (Axtell and Bartel, 2005).

One of the best known miR172 targets is *AP2*. Homologs and orthologs of *AP2* have been found in a variety of gymnosperms and angiosperms (Shigyo et al., 2006), and have also been detected in the fern *Ceratopteris thalictroides* (Axtell and Bartel, 2005). Phylogenies of both gymnosperm and angiosperm members of the *AP2* family suggest significant conservation of the target site of miR172 (Shigyo et al., 2006). Very few nucleotides differ between the miR172 binding sites of *AP2* genes from *Arabidopsis*, rice, maize and barley (Zhu and Helliwell, 2011). Moreover, *AP2* orthologs in these species have very specific expression patterns in inflorescences and floral primordia (Aukerman and Sakai, 2003; Chuck et al., 1998; Nair et al., 2010; Wollmann et al., 2010; Zhu et al., 2009).

The SPL transcription factors, which were first identified in *Antirrhinum majus*, contain an SPB-box region, bind to the promoter of the floral identity gene *SQUAMOSA* and were originally proposed to function on specifying flowers by activating genes that control floral meristem identity (Klein et al., 1996). Most members of *SPL* genes contain a site targeted by miR156. In *Arabidopsis*, 11 of the 17 *SPL* genes are targets of miR156, whereas in rice, 11 *SPL* genes are targeted by miR156 (Cuperus et al., 2011; Xie et al., 2006). SPB-box genes have been also found to be targeted by miR156 in the moss (Riese et al., 2007).

The targets of members of the miR166/165 family are *Class III HOEMEODOMAIN-LEUCINE ZIPPER (HD-ZIP III)* transcriptional factor genes (Floyd and Bowman, 2004). The *HD-ZIP III* genes are highly conserved in land plants, having been identified in dicotyledonous, monocotyledonous, gymnosperm, and moss species (Floyd and Bowman, 2004; Prigge and Clark, 2006). In addition, all of these homologs have the same miR166/165 binding site, indicating that miR166/165-mediated *HD-ZIP III* gene regulation is conserved in all land plants (Sakaguchi and Watanabe, 2012).

Members of the miR164 family are potentially capable of targeting the NAC family of transcription factor genes (Laufs et al., 2004). Currently, at least five NAC transcription factor genes are targeted by miR164 in *Arabidopsis*, including *CUP-SHAPED COTYLEDON1* (*CUC1*) and *CUC2*, both of which regulate plant development (Laufs et al., 2004; Kim et al., 2009; Raman et al., 2008). Other miR164 target genes have been also found in a variety of species, including *EgNAM1*and *PdNAM1* from palms (Adam et al., 2011) and *PaNAC01* from gymnosperms (Larsson et al., 2012).

Several auxin response transcription factors (ARFs) are regulated by miR160 and miR167. Five of the 23 *ARF* genes in *Arabidopsis* are targeted by these two miRNAs, with *ARF10*, *ARF16*, and *ARF*17 targeted by miR160, and *ARF6* and *ARF8* targeted by miR167 (Nagpal et al., 2005; Wu et al., 2006a, 2006b). Both *ARF6* and *ARF8* are also targeted by miR167 in rice (Yang et al., 2006).

The targets of miR319 are a subset of *TEOSINTE BRANCHED/CYCLOIDEA/PCF* (*TCP*) family of transcription factor genes. The targets of miR319 have been identified in *Arabidopsis*, rice, and sugarcane (Nag et al., 2009; Thiebaut et al., 2012).

Evolutionary conservation of miRNA regulatory programs during the floral transition

The floral transition is the most important phase during plant development because the proper timing of this transition is critical

Table 1
MiRNAs and their targets identified experimentally and their functions in flower development.

miRNA	Targets	Functions	species	References
miR156	*SPL3,4,5,9,10,15*	Floral transition	*Arabidopsis thaliana*	Wu and Poethig 2006, Gandikota et al. (2007), Schwarz et al. (2008), Wang et al., 2008; Wang et al. (2009), Wu et al. (2009), Yamaguchi et al. (2009)
	SBP1	Floral induction	*Antirrhinum majus*	Preston and Hileman (2010)
	tsh4;tga1	Bracts development, establish meristem boundaries	*Zea mays*	Hultquist and Dorweiler (2008), Chuck et al. (2010)
	OsSPL14	Panical branching and grain productivity (*SPL9* ortholog)	*Oryza sativa*	Xie et al. (2006), Miura et al. (2010), Jiao et al. (2010)
	SBP-box	Inflorescence and fruit development	*Solanum lycopersicon*	Zhang et al. (2011)
miR159	*AtMYB*	Flowering time and anther development	*Arabidopsis thaliana*	Achard et al. (2004)
	HvGAMYB	Anther development	*Hordeum vulgare*	Murray et al. (2003), Achard et al. (2004)
	LtGAMYB	Floral transition	*Lolium temulentum*	Gocal et al. (1999), Woodger et al. (2003), Achard et al. (2004)
	AvGAMYB	Flower development	*Avena sativa*	Woodger et al. (2003), Achard et al. (2004)
	OsGAMYB;OsGAMYB1	Anther development	*Oryza sativa*	Achard et al. (2004), Kaneko et al. (2004), Tsuji et al. (2006)
	Fa-GAMYB	Receptacle development	*Fragaria*	Csukasi et al. (2012)
miR160	*ARF10,16,17*	Floral organ development	*Arabidopsis thaliana*	Mallory et al. (2005), Liu et al. (2010)
miR164	*CUC1, 2*	Organ boundary formation; petal development; carpel closure	*Arabidopsis thaliana*	Laufs et al. (2004), Mallory et al. (2004), Baker et al. (2005), Jasinski et al. (2010)
	NAM	Establish flower primordia boundaries	*Petunia*	Souer et al. (1996)
	CUPULIFORMIS	Organ boundary formation	*Antirrhinum majus*	Weir et al. (2004)
	PaNAC01	Shoot apical meristem formation (*CUC2* ortholog)	*Picea abies*	Larsson et al. (2012)
	VvNAC1,2	Flower development	*Vitis vinifera*	Sun et al. (2012)
	NAM-related	Spikelet meristem and floral meristem development	*Oryza sativa*	Ooka et al. (2003), Adam et al. (2011)
	EgNAM1	Flower development	*Elaeis guineensis*	Adam et al. (2011)
	PdNAM1	Flower development	*Phoenix dactylifera*	Adam et al. (2011)
	GOBLET	Shoot apical meristems formation	*Solanum lycopersicon*	Berger et al. (2009)
miR165/ miR166	*PHB, PHV, REV, ATHB8,15*	Floral organ polarity	*Arabidopsis thaliana*	Kim et al. (2005), Williams et al. (2005), Jung and Park (2007)
	OsHB	Shoot apical meristems formation	*Oryza sativa*	Nogueira et al. (2007)
	RLD	The initiation and maintenance of the shoot apical meristems	*Zea mays*	Nagasaki et al. (2007)
	PpHB10	Shoot apical cell development	*Physcomitrella patens*	Prigge and Clark (2006)
miR167	*ARF6,8*	Gynoecium and stamen development	*Arabidopsis thaliana*	Nagpal et al. (2005), Wu et al. (2006)
	ARF8	Auxin signaling; anther development	*Oryza sativa*	Yang et al. (2006), Fujioka et al. (2008)
	unknown	Flower development	*Carya cathayensis*	wang et al. (2012)
miR169	*NF-YA*	Represses C Class activity in the perianth	*Antirrhinum, Petunia*	Cartolano et al. (2007)
miR172	*AP2*	Floral organ identity	*Arabidopsis thaliana*	Aukerman and Sakai (2003), Chen (2004), Wollmann et al. (2010)
	AP2-like (TOE1,2,3, SMZ, SNZ)	Flowering time	*Arabidopsis thaliana*	Aukerman and Sakai (2003), Schmid et al. (2003), Jung et al. (2007), Mathieu et al. (2009), Wu et al. (2009), Yant et al. (2010)
	SNB, OsIDS1	Branching and flower development	*Oryza sativa*	Lee and An (2012)
	Ids1,sid1	Inflorescence and floret development	*Zea mays*	Chuck et al. (2007b), Chuck et al. (2008)
	AP2a	Fruit development	*Solanum lycopersicon*	Karlova et al. (2011)
	RAP1	Flowering and tuberization	*Solanum tuberosum*	Martin et al. (2009)
	InAP2-like	Flower induction	*Ipomoea nil*	Glazińska et al. (2009)
	HvAP2	Lodicules development	*Hordeum vulgare*	Nair et al. (2010)
	AP2	Flower development, cycles of dormancy	*Actinidia*	Varkonyi-Gasic et al. (2012)
	CsatAP2	Floral organs development	*Crocus sativus*	Tsaftaris et al. (2011)
	Domestication gene Q	Domestication process	*Triticum aestivum*	Simons et al. (2006)
miR319	*TCP4*	Petal growth and development	*Arabidopsis thaliana*	Nag et al. (2009)

to ensure reproductive success (Huijser and Schmid, 2011). Flower initiation occurs in the SAM, which acts as a floral inducer and triggers the floral transition network. In *Arabidopsis*, when signals from flower-promoting pathways reach the SAM, the vegetative meristem first acquires an inflorescence meristem (IM) identity. The IM then gives rise to the reproductive organs after generating the FM on its flank. In monocotyledonous species, the primary IM produces several lateral branches. Each branch meristem gives rise to spikelet meristems (SMs), each of which eventually differentiates into the FM (Liu et al., 2009a, 2009b; Barton, 2010).

Genetic and molecular analyses in *Arabidopsis* and other plants have identified multiple interdependent genetic pathways that control the timing of the floral transition. Some key components are thought to be very important in the floral transition network. One class of key components is the group of floral meristem identity genes, such as *LEAFY* (*LFY*) and *APETALA1* (*AP1*), which promote the differentiation of SAM primordia into FMs (Liljegren et al., 1999; Wigge et al., 2005). The other class comprises floral integrator genes, such as *SUPPRESSOR OF OVEREXPRESSION OF CO* (*SOC1*), which together with *AGAMOUS-LIKE 24* (*AGL24*), promote the expression of the floral meristem identity genes *LFY* and *AP1* (Amasino, 2010; Schmid et al., 2003; Wigge et al., 2005). Moreover, the small mobile protein Flowering Locus T (FT) acts as a florigen, and plays a key role by activating *SOC1* to promote flowering together with the meristem-specific bZIP transcription factor FD (Abe et al., 2005; Amasino, 2010; Srikanth and Schmid, 2011).

The key components of floral transition networks mentioned above are regulated by various environmental and endogenous cues, such as photoperiod, temperature, gibberellins (GAs), and vernalization. Recent studies have shown these key genes or proteins are also regulated by endogenous miRNAs (Poethig, 2009; Nag and Jack, 2010). It has become increasingly clear that three miRNAs with a high degree of evolutionary conservation – miR156, miR172, and miR159 – directly or indirectly regulate those components that are critical to the regulation of flowering (Fig. 1 A).

MiRNA regulatory programs in leaves and the SAM in floral transition of Arabidopsis

The floral transition occurs in both leaves and the SAM. Leaves receive inductive environmental cues, and the subsequent production of the floral promoter FT triggers the establishment of the regulatory network that governs the timing of flowering. After being transported to the SAM, FT prompts a genetic network to activate the floral meristem identity genes, which culminates in differentiation of the SAM into the FM. Different regulatory circuits, which comprise miR156, miR172, and miR159 together with their targets, control the floral transition in leaves and the SAM (Fig. 3).

In leaves, miR156 and miR172 regulate the production of FT through a complex regulatory circuit (Fig. 3). Expression of the *FT* gene is activated by GIGANTEA (GI) and COSTANTS (CO) in leaves following photoperiodic induction in *Arabidopsis* adult plants (Amasino, 2010). However, the FT protein is also regulated by the targets of miR156 and miR172. MiR172 and its *AP2-like* targets use a CO-independent mechanism to regulate *FT* (Jung et al., 2007). *AP2-like* genes, such as *TARGET OF EAT1* (*TOE1*), *TOE2*, *TOE3*, *SCHLAFMUTZE* (*SMZ*), and *SCHNARCHZAPFEN* (*SNZ*) act as repressors of *FT* (Aukerman and Sakai, 2003; Jung et al., 2007; Schmid et al., 2003). Elimination of all *AP2-like* genes results in extremely early flowering, similar to the phenotype caused by overexpression of miR172 (Yant et al., 2010). Chromatin immunoprecipitation (ChIP) analysis revealed that *FT* is directly targeted by SMZ, and that changes in the levels of *FT* accumulation caused by alteration of the levels of either *SMZ* or miR172 occur independently of CO (Mathieu et al., 2009). Furthermore, the expression pattern of miR172 is correlated with that of miR156. The miR156

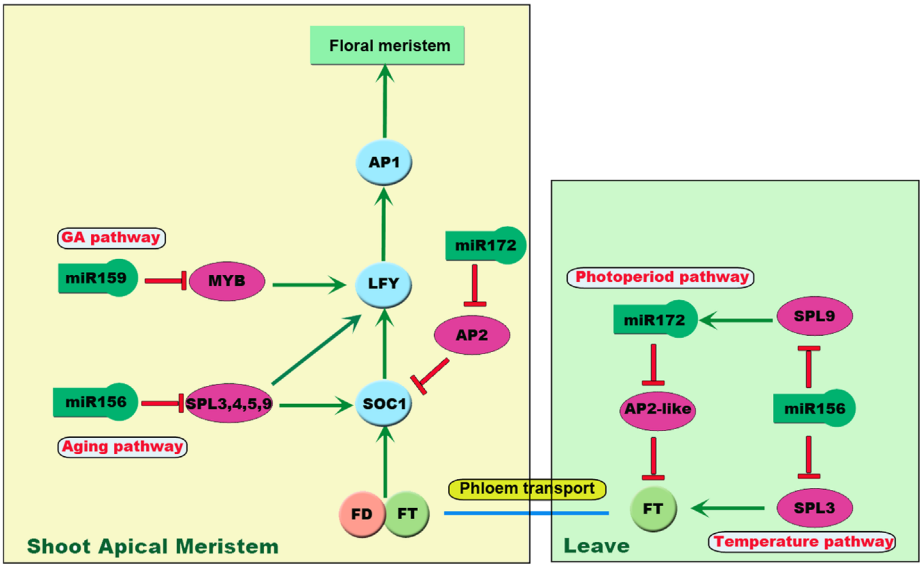

Fig. 3. A schematic diagram of the molecular mechanisms of miR156, miR172, miR159 and their targets regulating floral transition in leaves and the apical shoot meristem in *Arabidopsis*. A regulatory circuit consisting miR156, miR172 and their targets exist in leaves to control the expression of the florigen FT. *AP2-like* genes targeted by miR172 mediate the induction of flowering by photoperiod. MiR172 repress expression of *AP2-like* genes, which negatively regulate *FT*. MiR172 expression is positively regulated by certain *SPL* genes, which is repressed by miR156. MiR156-*SPL* module is also positive regulators of *FT* through temperature pathway. The FT protein is transported through the phloem to the shoot apical meristem. In the shoot apical meristem, miRNAs can be involved in floral meristems initiation by directly regulating the floral meristem identity genes through different pathway. FT interacts with the bZIP transcription factor FD and coordinately they upregulate *SOC1* and *AP1*, then give rise to identity of floral meristem. *SPLs* targeted by miR156 can active *SOC1* and *LFY*, through age pathway. *MYBs* targeted by miR159 regulate *LFY* through gibberellins pathway. *AP2* gene also participates in *SOC1* repression in the shoot apical meristem. Green lines with an arrow represent promotion, and red ones with a perpendicular bar represent repression. Ovals represent genes or proteins involved in floral transitions, and purple ones represent targets of miRNAs.
Abbreviations used in the figure: FT, Flowering Locus T; SOC1: SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1; AGL24, AGAMOUS-LIKE 24; LFY, LEAFY; AP1, APETELA1; AP2-like, APETALA2-like; SPL, SQUAMOSA PROMOTER BINGDING PROTEIN-LIKE.

target *SPL9* promotes expression of miR172 (Wu et al., 2009). In addition, the miR156-*SPLs* module can directly regulate *FT* (Kim et al., 2012; Lee et al., 2010). Overexpression of miR156 in leaves delays flowering at a lower ambient temperature (16 °C), and this is associated with a reduced abundance of *FT* transcripts (Kim et al., 2012; Lee et al., 2010). The SPL3 protein binds directly to *FT* to regulate flowering (Kim et al., 2012). However, the miRNA-mediated temperature-sensitive pathway appears to be independent of known temperature-sensitive pathways. *SHORT VEGETATIVE PHASE* (*SVP*), *FLOWERING LOCUS C* (*FLC*), *FLOWERING LOCUS M* (*FLM*) and other components in known temperature-sensitive pathways (Balasubramanian et al., 2006; Lee et al., 2007a, 2007b), have no correlation with miR156 or *SPLs*. These findings suggest the existence of a feedback loop in the miR156/miR172-mediated module that controls flowering.

In the SAM, the targets of miR156, miR172 and miR159 participate in the activation of floral meristem identity genes (Fig. 3). The miR156-*SPL* module promotes the floral transition through an age-dependent pathway. MiR156 targets a subset of *SPLs*, which were originally proposed to promote flowering (Klein et al., 1996). In young *Arabidopsis* seedlings, miR156 levels are high and levels of *SPLs* are low. As development proceeds, the decline of miR156 levels and the increase of *SPLs* in the SAM lead to the activation of floral meristem identity genes (Wang et al., 2009; Wu et al., 2009). *SPLs* activate expression of the floral meristem identity genes *LFY* and *AP1* and the floral integrator gene *SOC1* (Yamaguchi et al., 2009; Wang et al., 2009). With decreasing miR156 activity, flowering can be promoted through the photoperiod-dependent FT/FD pathway, and increasing levels of *SPLs* might trigger the floral switch (Schmid et al., 2003; Wang et al., 2009). A continuing increase in the activities of *SPLs* will cause plants to flower even without activation of the FT/FD pathway (Wang et al., 2009). This change in levels of miR156 and *SPLs* appears to be independent of several known floral regulators, such as temperature, vernalization, and GAs. The miR156-*SPLs* module thus seems to define an age-dependent pathway to induce flowering. MiR159-*MYB* modulates the regulation of the floral transition by GA through targeting the GA-specific transcriptional regulator *GAMYB*. The GA signaling pathway plays an important role in promoting flowering during short-day photoperiods (Wilson et al., 1992; Simpson and Dean, 2002). *Arabidopsis AtMYB33* is a GA-specific transcriptional regulator that regulates the floral initiation by activating *LFY* in the GA signaling pathway (Blázquez and Weigel, 2000; Gocal et al., 2001). *MYB33* expression is strongly repressed by miR159 (Achard et al., 2004; Allen et al., 2007; Alonso-Peral et al., 2010). Overexpression of miR159 resulted in a delay in flowering in short-day photoperiods that was associated with a reduction in the levels of *MYB33* and of *LFY* (Achard et al., 2004). In the SAM, the miR172 target protein AP2 can directly repress *SOC1*, thus adding another level of regulation downstream or in parallel of FT (Yant et al., 2010). Similarly, both SMZ and AP2 bind to their own promoter sequences as well as those of other miR172 targets, and regulate their expression in a rather complex negative feedback network (Mathieu et al., 2009; Yant et al., 2010).

In summary, miR156, miR172, and miR159 and their targets are important components of the complex network of floral transition regulation. Together, these miRNA-mediated pathways regulate the transcription of a small number of key integrator genes, including *FT*, *SOC1*, and *LFY*. In leaves, *FT* is negatively regulated by *AP2-like* genes, which are targets of miR172, and positively regulated by *SPL3*, which is a target of miR156. *SPLs* are positive regulators of miR172. In the SAM, *SOC1* is positively regulated by miR156-*SPLs* and negatively regulated by miR172-*AP2-like* genes. *LFY* is a common target of the miR156-*SPLs* and miR159-*MYB33* modules (Fig. 3). Overall, three miRNA-mediated pathways are independent of other pathways that determine the timing of the floral transition. Whereas the miR156-*SPLs* module participate in the aging pathway as well as the ambient temperature-responsive pathway, the miR172-*AP2-like* module participates in the photoperiodic pathway, and the miR159-*MYB33* module participates in GA signaling (Fig. 3).

The meristem transition is regulated by miR156 and miR172 in monocotyledonous species

Recent studies from rice and maize have shown miR156 and miR172 control the conversion of SMs to FMs to ensure the initiation of floral organ primordia (Fig. 1a2). Maize contains at least 17 genes that encode SBP-box proteins and are potential targets of miR156 (Hultquist and Dorweiler, 2008). The maize *Corngrass1* (*Cg1*) mutant, which carries a *STONER* retrotransposon insertion in the upstream regulatory region of the miR156 gene, causes overexpression of miR156 and reduces the number of branches in tassels and increased tilling (Chuck et al., 2007a). The *teosinte glume architecture1* (*tga1*) gene, which encodes an SBP-box-containing protein, is also expressed at a lower level in the *Cg1* mutant than in its wild-type counterpart, and mutation of *tga1* has been implicated in the domestication of maize (Chuck et al., 2007a; Hultquist and Dorweiler, 2008; Wang et al., 2005). Another SBP-box gene, *tasselsheath4* (*tsh4*), is also a target of miR156, and is known to regulate the development of bracts and meristem boundaries (Chuck et al., 2010). Some *AP2-like* orthologous genes are targets of miR172. These include *Indeterminate Spikelet1* (*Ids1*) and *Sister of Indeterminate Spikelet 1* (*Sid1*), which control spikelet meristem fate in maize (Chuck et al., 1998, 2007b, 2008). Additional spikelets and florets are formed on branches in the maize mutant *tassel seed 6* (*ts6*), which carries a mutation in the miR172 binding site of *Ids1*. A very similar phenotype is observed in *ts4*, which carries a mutation in miR172e (Chuck et al., 2007b). Reduced expression of *Sid1* delays the conversion of SMs to FM identity. An *ids1* and *sid1* double mutant forms many bracts from SMs, and florets are never initiated (Chuck et al., 2008). The inflorescence and floret defects caused by mutations in these miRNA genes or target genes suggest a central role for miR156 and miR172 in determining the identities of maize inflorescence or FMs.

In rice, 11 *OsSPL* genes are putative targets of miR156. Overexpression of miR156 causes severe dwarfism, strongly reduced panicle size, and delayed flowering (Xie et al., 2006). The miR156 target *OsSPL14* gene regulates panicle branching in the inflorescences and directly affects grain yield in rice (Jiao et al., 2010; Miura et al., 2010). Overexpression of miR172 delays the transition from SM to FM, changing the numbers and identities of floral organs (Zhu et al. 2009). The wheat domestication gene Q, which is also a miR172 target and is orthologous to maize *Ids1*, affects several domestication-related traits, including spike compactness and glume shape (Simons et al., 2006).

From these results it appears that miR156 and miR172 also participate in the floral transition in monocotyledonous species. This indicates that miRNA-mediated pathways that regulate the floral transition are conserved in flowering plants.

The role of miRNAs in the floral patterning

After completion of the floral transition, different floral organ identity genes are activated by floral meristem identity genes, and thus convert FMs into floral organ primordia. The formation of floral organs relies on the effects of floral organ identity genes, and the establishment of boundaries. To date, two miRNAs are known to be involved in floral organ formation. MiR172 controls the inner

whorl organ formation by restricting the expression of the *AP2* gene, whereas the miR164 gene targets *NAM*-related genes to establish boundaries between whorls of organs, and also between organs within whorls, which define the sizes of the primordia and the resulting floral organs.

The role of miR172 in perianth identity in Arabidopsis

The floral organ primordia occur in successive whorls in most dicotyledonous species. In *Arabidopsis*, these comprise a whorl of four sepal primordia, a whorl of four petal primordia, followed by a whorl of six stamen primordia, and finally two carpel primordia. The activation of ABC model genes specifies floral organ identity (Causier et al., 2010).

AP2 is an A-class gene that is well known for its role in specifying perianth development through interacting with other A- or B-class genes, and acts mutually antagonistic with the C-glass gene *AGAMOUS* (*AG*) (Jofuku et al., 1994). It is puzzling that *AP2* transcripts were reported to accumulate throughout all of the floral whorls, rather than being limited to the outer two whorls, and this is not consistent with the role of *AP2* in specifying perianth identity (Jofuku et al., 1994). However, it has recently been reported that *AP2* expression is actually restricted to the outer two whorls (Chen, 2004; Zhao et al., 2007; Wollmann et al., 2010). The key regulator for this event is referred to miR172, which repress *AP2* expression at the post-transcriptional level (Chen, 2004). In situ hybridization experiments show that miR172 and *AP2* expression are largely complementary, with miR172 in the inner floral whorls and *AP2* in the outer floral whorls (Fig. 1b1) (Chen, 2004; Zhao et al., 2007; Wollmann et al., 2010). Therefore the function of AP2 protein is restricted to the outer whorls and it specifies the formation of the primordia that give rise to sepals and petals. However, there is transient overlap between miR172 and *AP2* in second and third whorl primordia from stage 3 onwards. It would appear that miR172 is not sufficient to fully restrict *AP2* activity, and thereby specifying the boundary between perianth and reproductive organs (Wollmann et al., 2010).

Two upstream regulators of miR172, LEUNIG (LUG) and SEUSS (SEU), were identified recently. These proteins bind to the *AG cis*-regulatory elements to repress AG transcription in the two outer whorls (Franks et al., 2002; Sridhar et al., 2004). The SEU-LUG co-repressor complex also negatively regulates miR172 expression in the outer whorls, cooperating with *AP2* (Grigorova1 et al., 2011).

The role of miR172 in the development of lodicules in monocotyledous species

Lodicules in the grasses appear to be structurally homologous to petals in *Arabidopsis* (Bommert et al., 2005). Lodicules are small structures at the base of the carpels that act to open the palea and lemma for anthesis. The involvement of miR172 in the development of lodicule was reported from rice and barley (Fig. 1b2).

Overexpression of miR172 in rice increases the number of lodicules and results in the enlargement of lodicules, which prevent the closing of spikelets after flowering (Zhu et al., 2009). The *SUPERNUMARY BRACT* (*SNB*) and *Oryza sativa INDETERMINATE SPIKELET1* (*OsIDS1*) genes are involved in the development of lodicules. They are homologous to *AP2* and are targeted by miR172. The *snb* or *osids1* single mutant, as well as their double mutants, have abnormal lodicules (Lee and An, 2012; Lee et al., 2007a, 2007b). *SNB* and *IDS1* might regulate the development of lodicules by either activating lodicule-specifying genes or repressing lemma/palea-specifying genes (Lee and An, 2012).

In barley, *CLEISTOGAMY1* (*Cly1*) is an *AP2* transcription factor gene regulated by miR172 (Nair et al., 2010). Mutation within the miR172 binding site of *Cly1* causes defective lodicule development, which prevents the lemma and palea from opening owing to the failure of lodicules to swell, which in turn causes self-pollination (Nair et al., 2010).

Establishment of boundaries by miR164

The *NO APICAL MERISTEM* (*NAM*)-related genes encode members of the plant-specific NAC family of transcription factors, which help to define morphogenetic boundaries (Aida et al., 1997; Souer et al., 1996). The *Arabidopsis* genes *CUC1, CUC2*, and *CUC3* (Takada et al., 2001; Vroemen et al., 2003) belong to the *NAM* gene family, and are involved in the establishment and maintenance of the shoot apical and axillary meristem during floral development to control floral organ formation (Aida and Tasaka, 2006). The *CUC1* and *CUC2* genes are targets of miR164 (Larue et al., 2009; Laufs et al., 2004; Mallory et al., 2004; Sieber et al., 2007), whereas *CUC3* is not (Vialette-Guiraud et al., 2011). Reduced expression of the *CUC1* and *CUC2* genes causes abnormal cellular proliferation within the boundaries of sepal primordia during the early phases of the sepal boundary development. In general, the enlargement of the sepal boundary causes defective flower phenotypes, such as the fusion of sepals and reductions of petals (Laufs et al., 2004). MiR164c can act independently to control the petal number by regulating the accumulations of *CUC1* and *CUC2* transcripts at the boundaries between petal primordia (Baker et al., 2005). *RABBIT EARS* (*RBE*), which encodes a C2H2 zinc finger transcriptional repressor, was recently identified as an upstream of regulator of miR164 that controls floral organogenesis (Huang et al., 2012).

Besides their roles in sepal and petal boundary development, miR164 and its targets are thought to be involved in carpel fusion (Jasinski et al., 2010; Larue et al., 2009; Nahar et al., 2012; Sieber et al., 2007). Carpel fusion is an important event in the evolution of angiosperms. MiR164 mutants show some defects in carpel fusion in *Arabidopsis* (Baker et al., 2005; Sieber et al., 2007). The ANA grade angiosperm, including Amborellales, Nymphaeales and Austrobaileyales, represents the three earliest lineages of extant angiosperm (Bremer et al., 2009). Jasinski et al. (2010) have shown that miR164 and *CUC* genes are associated with carpel closure in the ANA grade species *Amborella trichopoda* and *Cabomba aquatica*, indicating a potential mechanism for the initial evolution of closed carpels in early flowering plants.

The NAC-domain genes of many dicotyledonous species contain a potential miR164-binding site. Most *Petunia nam* mutants lack the SAM and die at the seedling stage (Souer et al., 1996). *Antirrhinum* Cupuliformis (CUP) is homologous to the *Petunia* NAM and *Arabidopsis* CUC proteins. The *cup* mutants are defective in the SAM formation (Weir et al., 2004). In tomato, the *NAM* ortholog *GOBLET* and miR164 are expressed in complementary domains in the SAM (Berger et al., 2009). Moreover, the *NAM*-related genes from some monocotyledonous species (e.g., rice and palm) and gymnosperm species also possess a miR164-binding site (Adam et al., 2011; Larsson et al., 2012; Li et al., 2010). The homologous NAM gene from oil palm (*Elaeis guineensis*) is *EgNAM1*. The observation that transgenic 35S-*EgNAM1 Arabidopsis* plants have similar leaf and inflorescence phenotypes to those of plants that overexpress *CUC2* indicates that the *NAM*-related genes of palm are functionally equivalent to *AtCUC2* (Adam et al., 2011). Moreover, the observation that the expression of *PaNAC01*, a *CUC*-like homolog in *Picea* increases dramatically as early embryos start to differentiate (Larsson et al., 2012), indicates that miR164-dependent regulation of *NAM*-related genes is associated with SAM differentiation in gymnosperms.

The conservation of the miR164 and *NAM*-related gene expression patterns observed in dicotyledonous, monocotyledonous, and gymnosperm species suggests that the regulation of organ

boundary establishment and SAM differentiation is likely to be evolutionarily conserved throughout seed plants. MiR164 and their target genes are identified in some species that occupy key phylogenetic positions, and the phylogeny of the miR164 family indicates that miRNAs and their targets may play key roles in the evolution of carpel closure (Jasinski et al., 2010; Vialette-Guiraud et al., 2011).

The role of miRNAs in the development of floral organs

The evolutionary conserved miRNAs miR159, miR160, miR166/165, miR167, miR169, and miR319 regulate the development of floral organs, including the growth and differentiation of sepals, petals, anthers, and carpels (Fig.1c1 and c2).

MiR159 regulates anther development

Angiosperms share a conserved program that directs anther development. MiR159 and its targets the *GAMYB*-related genes are required for normal anther development in *Arabidopsis*, rice, and barley (Achard et al., 2004).

GAMYB was first identified as a positive regulator of GA signaling in barley aleurone cells during seed development (Gubler et al., 1999). Subsequent studies revealed that *GAMYB* also functions in flower development (Murray et al., 2003). In barley, overexpression of *HvGAMYB* results in decreased anther length and causes male sterility (Murray et al., 2003). *GAMYB* genes were also identified in other species, such as *Arabidopsis*, rice, *Avena sativa*, and *Lolium temulentum* (Achard et al., 2004; Tsuji et al., 2006; Woodger et al., 2003). The expression and function of *GAMYB* genes appear to be highly conserved (Achard et al., 2004; Kaneko et al., 2004; Millar and Gubler, 2005). The *GAMYB* and *GAMYB*-like genes from *Arabidopsis*, rice, *A. sativa*, and *L. temulentum* share a conserved miR159-binding site (Achard et al., 2004; Millar and Gubler, 2005; Tsuji et al., 2006).

Arabidopsis MYB33 and *MYB65* belong to the *GAMYB*-like family. The expression of *AtMYB33* is restricted to young anthers. Tapetum hypertrophy and pollen abortion are found in the *Arabidopsis myb33 myb65* double mutants (Millar and Gubler, 2005). Overexpressing miR159 decreases levels of *AtMYB33*, and causes anther defects, male sterility, and delayed flowering (Achard et al., 2004; Schwab et al., 2005). Alonso-Peral et al. (2010) have found that miR159 acts as a molecular switch that restricts the expression of *MYB33* and *MYB65* to anthers.

In rice, three genes that encode *GAMYB*-like transcription factors were found to contain a miR159-binding site (Tsuji et al., 2006). As in *Arabidopsis*, *OsGAMYB* expression is also anther-specific and is negatively correlated with miR159 expression (Aya et al., 2009; Tsuji et al., 2006). Loss-of-function mutations of *OsGAMYB* caused defects in anthers and pollen (Kaneko et al., 2004). The expression of *OsGAMYB::GUS* in both the tapetum and young microspores suggests that *GAMYB* may also directly regulate microspore development (Aya et al., 2009). More recently, the interaction between miR159 and *GAMYB* was reported in strawberry, where their functions correlate with receptacle development (Csukasi et al., 2012).

Strong similarities in anther development between *Arabidopsis*, rice, and barley, and the homologies between the transcription factors that regulate the process in different species, suggest the existence of a conserved anther developmental program within angiosperms.

MiR166/165 regulates the SAM and floral organ polarity

The miR166/165 group includes two miRNAs, miR166 and miR165. Both miRNAs were identified in *Arabidopsis*, but only miR166 has been identified in other species (Jones-Rhoades and Bartel, 2004). Mature miR165 and miR166 sequences in *Arabidopsis* differ by only a single nucleotide (Reinhart et al., 2002), and both miR166 and miR165 target the same set of the *HD-ZIP III* genes that perform the same functions (Zhou et al., 2007). MiR166/165 targets the *HD-ZIP III* transcription factor genes *ATHB15*, *ATHB8, REVOLUTA* (*REV*), *PHABULOSA* (*PHB*), and *PHAVOLUTA* (*PHV*) (Floyd and Bowman, 2004). Comprehensive genetic studies in *Arabidopsis* have shown miR166/165 and their targets play an important role in shoot apical and lateral meristem formation, organ polarity, and vascular development (Kim et al., 2005). MiR166-mediated repression of *ATHB15* may have a role in apical meristem formation as well as in vascular development in inflorescence stems. Overexpression of miR166 or miR165 resulted in reduced floral organs. The gain-of-function miR166a mutant *meristem enlarged1* (*men1*) and a miR166 activation-tagged mutant *jabba-1D* (*jab-1D*) have fasciated inflorescence stems, drastically reduced levels of *ATHB15* compared with wild-type plants, and defective vascular differentiation and radial patterning (Kim et al., 2005; Williams et al., 2005). Overexpression of miR165 reduces levels of all five *HD-ZIP III* genes, and causes developmental defects in the SAM (Zhou et al., 2007). The *HD-ZIP III* family genes are also required for organ polarity. The *REV* gene is expressed in developing vascular tissues and adaxial cells of floral organs, the FM, and at presumptive FM initiation sites (Prigge et al., 2005). MiR166/165 genes regulate SAM formation and floral development by acting in parallel with the *WUSCHEL* (*WUS*)–*CLAVATA* (*CLV*) pathway (Jung and Park, 2007). The individual MIR166/165 gene expression showed distinct temporal and spatial expression patterns in different plant organs, suggesting that miR166/165 might differentially regulate target genes during plant development (Jung and Park, 2007).

Recent studies have shown miR166/165 regulate *HD-ZIP III* transcripts through ARGONAUTE10 (AGO10) proteins (Liu et al., 2009a, 2009b; Zhu et al., 2011). *AGO10* modulates the SAM maintenance and the establishment of organ polarity by genetic repression of miR165/166 expression (Liu et al., 2009a, 2009b). The prolonged floral meristem maintenance associated with the *ago10* loss-of-function mutation suggests that AGO10 competes with AGO1 for binding to miR166/165 (Zhu et al., 2011). AGO10 and miR166/165 are also involved in floral stem cell termination. The reduction of the *HD-ZIP III* genes by rendering them resistant to miR166/165 can prolong the activity of floral stem cell (Ji et al., 2011).

Downregulation of the *HD-ZIP III* genes of rice and maize by miR166 affects leaf polarity and defects in the embryonic SAM (Nagasaki et al., 2007; Nogueira et al., 2007). There are three copies of miR166 genes but no miR165 in the lycopod *Selaginella* (Axtell et al., 2007).The homologous *HD-ZIP III* genes in *Selaginella* are expressed adjacent to the shoot apical cell region (Prigge and Clark, 2006).

The miR166/165 complementary sequence is highly conserved in mRNAs of *HD-ZIP III* genes and their homologs from dicotyledonous, monocotyledonous, and moss species (Floyd and Bowman, 2004; Prigge and Clark, 2006). It is therefore likely that miR166-mediated *HD-ZIP III* gene repression is highly conserved in all land plants.

MiR167 and miR160 regulate ARFs to control the formation of floral organs

ARFs regulate the expression of a large set of auxin-responsive genes by binding to the auxin-response elements in their promoters (Guilfoyle and Hagen, 2007; Mockaitis and Estelle, 2008). Some ARF proteins have been shown to regulate floral organ formation and to be targeted by miRNAs (Chapman and Estelle,

2009; Mallory et al., 2005; Wu et al., 2006a, 2006b). Of the known *ARF* genes, *ARF6* and *ARF8* are targeted by miR167, whereas *ARF10*, *ARF16*, and *ARF17* are targeted by miR160 (Mallory et al., 2005; Wu et al., 2006a, 2006b).

ARF6 and *ARF8* are essential for both ovule and anther development (Nagpal et al., 2005; Wu et al., 2006a, 2006b). Flowers of *arf6 arf8* double loss-of-function mutants show some defects in anther and gynoecium development, such as short stamens, anthers indehiscence, and defective ovule integuments (Ru et al., 2006; Wu et al., 2006a, 2006b; Yang et al., 2006). Overexpression of miR167 causes defects in anther dehiscence and failure to release pollen owing to reduced levels of *ARF6* and *ARF8* transcripts. These results suggest that the downregulation of *ARF6*/*ARF8* by miR167 is critical for pollen development. In rice, miR167 accumulates to high levels during the late stage of anther development, indicating its role in regulating anther development is conserved (Fujioka et al., 2008).

Several studies have shown that miR160 negatively regulates *ARF10*, *ARF16*, and *ARF17* (Mallory et al., 2005; Liu et al., 2010). A loss-of-function mutant *floral organs in carpel* (*foc*) with a *Ds* transposon insertion in the 3′ regulatory region of miR160, exhibits some defects of flowering, such as the formation of irregularly shaped flowers and floral organs inside siliques, as well as reduced fertility (Mallory et al., 2005; Liu et al., 2010). In the *foc* mutant, *ARF10*, *ARF16*, and *ARF17* transcripts are all present at levels higher than those in wild-type plants owing to reduced expression of miR160.

MiR169 regulates the C-class gene to control the development of reproductive organs

The expression of C-class genes is repressed by miR169. Cartolano et al. (2007) demonstrated that *FISTULATA* (*FIS*) in *Antirrhinum majus* and *BLIND* (*BL*) in *Petunia hybrida* encode miR169. *FIS* and *BL* restrict C-class gene activity to the inner two floral whorls to specify the identities of the reproductive organs in the flower. Loss-of-function mutants of *FIS* and *BL* produce stamenoid petals in their second whorls, which is indicative of abnormal C function in the second whorl. Nonetheless, there is no miR169 target site in C-class genes. MiR169 targets members of the NF-YA transcription factor gene family (Davies et al., 1999; Jones-Rhoades and Bartel, 2004). Given that NF-YA transcription factors can activate C-class genes, miR169 are anticipated to repress the expression of C-class genes by post-transcriptional repression of *NF-YA* members. Although a miR169/*NF-YA* module exists in *Arabidopsis*, the function of restricting class C gene expression has not been detected (Cartolano et al., 2007).

MiR319 regulates petal development

It is interesting that miR159 and miR319 share 17 identical nucleotides in *Arabidopsis*, and that the two miRNAs evolved from a common ancestor (Li et al., 2011). However, given that miR159 and miR319 have distinct expression patterns and target distinct genes, they have different functions (Palatnik et al., 2007). As mentioned above, miR159 targets several *GAMYB* transcription factor genes involved in floral initiation and anther development, whereas miR319 targets a subset of *TCP* transcription factor genes that control leaf and flower growth (Nag et al., 2009; Palatnik et al., 2007). A loss-of-function mutant of miR319 in *Arabidopsis* showed some defects in petal and stamen development, such as narrower and shorter petals and impaired anther formation (Nag et al., 2009). Plants with high TCP activity also suffer impaired development of floral organs in *Arabidopsis* (Koyama et al., 2007; Nag et al., 2009; Sarvepalli and Nath, 2011).

Conclusions and future perspectives

This review summarizes recent insights into the essential roles that miRNAs play in flower development. Nine highly conserved miRNA families have been implicated in regulating flower development. Most show considerable conservation among all most land plants at the levels of sequence conservation and target identities. Analysis of the level of conservation of miRNA-regulated systems in a variety of plant species reveals that miRNAs and their targets affect a broad spectrum of flower developmental programs, and that their regulatory modules are also broadly conserved across all angiosperms (Fig. 1). MiRNAs and their targets are key components in complex networks that control flower development. The interactions between miRNAs, their targets, regulatory factors that act upstream of miRNAs, and other genes involved in flower development have been extensively characterized in *Arabidopsis*, rice, and maize. However, the conserved miRNA-target modules that are believed to control flowering require further investigation in other species. Several questions about the evolution of these miRNAs remain to be established. The origin(s) and evolution of miRNA-target regulatory systems are not understood, and even less is known about the phylogenetic aspects of miRNA functions, the evolutionary relationships between miRNAs and their targets, and the factors that drive the co-evolution of miRNAs and their targets.

We anticipate that future studies will identify more miRNAs and their targets in non-model species, especially those that belong to key evolutionary lineages that include basal angiosperms, gymnosperms, ferns and mosses. Moreover, it is important to examine the molecular mechanisms that support the regulatory roles of miRNAs, and to obtain a more comprehensive understanding of the evolution of miRNA-mediated regulatory pathways. Research in this area will undoubtedly help us to unravel the evolutionary history and level of conservation of the miRNA-target pathways that regulate growth and development.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ydbio.2013.05.009.

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