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The control of developmental phase transitions in plants

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Summary

Plant development progresses through distinct phases: vegetative growth, followed by a reproductive phase and eventually seed set and senescence. The transitions between these phases are controlled by distinct genetic circuits that integrate endogenous and environmental cues. In recent years, however, it has become evident that the genetic networks that underlie these phase transitions share some common factors. Here, we review recent advances in the field of plant phase transitions, highlighting the role of two microRNAs – miR156 and miR172 – and their respective targets during these transitions. In addition, we discuss the evolutionary conservation of the functions of these miRNAs in regulating the control of plant developmental phase transitions.

Key words: Phase change, Phase transition, microRNA, miR156, miR172, AP2, SPL, Transcription factor, Flowering

Introduction

The life cycle of flowering plants can be considered as a succession of distinct growth phases (Fig. 1), and the transition between these phases is dependent on developmental genetic programs that are triggered and modulated by both environmental and endogenous stimuli. Following germination, and before they become competent to flower and reproduce, the shoots of most plants pass through a phase of vegetative growth. During this period, plants generally rapidly increase their photosynthetic capacity and their size and mass. This vegetative mode of growth can be further divided into a juvenile and an adult vegetative phase. Although generally less conspicuous in annuals such as *Arabidopsis*, these phases are often recognizable by a changed growth pattern and body form, particularly in perennial species. For example, in some cephalium-bearing cacti, the different phases resemble graftings of different plant species (Mauseth, 2006). It is normally only during the adult vegetative phase that plants are capable of forming reproductive organs, and day length-dependent plants can be induced to flower by photoperiodic induction during this phase. During the juvenile-to-adult phase transition (see Glossary, Box 1), plants thus acquire reproductive competence. Simultaneously, changes in multiple traits, such as leaf size and shape, internode length and trichome distribution, result in the appearance of both early (juvenile) and mature (adult) shoots on the same plant, a condition known as heteroblasty (see Glossary, Box 1) (Goebel, 1889; Poethig, 1990; Poethig, 2010).

Eventually, another phase transition, known as the reproductive phase change (see Glossary, Box 1), occurs. During this transition, plants switch from vegetative to reproductive growth, and the vegetative shoot apical meristem (SAM, see Glossary, Box 1) takes on an inflorescence meristem (IM) identity. The developmental fate of the IM, i.e. its conversion into a floral meristem (FM) or its production of lateral meristems that may convert to FMs, is species specific and determines the type of inflorescence formed. As in other species, this transition is very obvious in *Arabidopsis*, and there is thus a great deal of knowledge regarding the molecular control of reproductive phase change and the determination of meristem identity (Adrian et al., 2009; Amasino, 2010; Srikanth and Schmid, 2011). The correct timing of the transition to flowering is of utmost importance to all plants, as it may have a strong impact on fitness. For example, flowering should take place when the climatic conditions are suitable and sufficiently reliable to allow completion of the process with the successful dispersal of seeds. In the case of non-self-fertile species, flowering also needs to be synchronized within a population and with the occurrence of potential pollinators.

The transition to flowering is under the control of a complex genetic network that integrates information from various endogenous and environmental cues (Amasino, 2010). Genetic analyses, mostly conducted in *Arabidopsis* and rice, have identified numerous genes that participate in the regulation of flowering. Endogenous factors that regulate reproductive phase change include hormones and carbohydrate assimilates, such as gibberellins and sugars (Corbesier et al., 1998; Moon et al., 2003; Ohto et al., 2001; Seo et al., 2011). Among the environmental factors that may affect plant growth, only a few seem to be specifically monitored to control flowering (Amasino, 2010; Srikanth and Schmid, 2011). The most important of these are temperature, including the ambient growth temperature a plant experiences throughout its life, as well as prolonged periods of low temperature (which cause vernalization, see Glossary, Box 1), and day length (photoperiod). Eventually, the activities of the flower inductive pathway genes converge on a small number of so-called floral integrators. Once the expression of these integrators exceeds a threshold, the plant initiates flowering, which is generally an irreversible process (Tooke et al., 2005). Besides being important to ensure the reproductive success of a species, the control of the vegetative-to-reproductive phase transition is also of considerable agronomical importance. For example, premature flowering usually results in reduced biomass and seed set. Similarly, prolonged vegetative growth might lead to an increase in biomass but at the same time often results in reduced seed number and seed filling (Demura and Ye, 2010).

In recent years, it has become increasingly clear that the networks that control the juvenile-to-adult phase transition and the reproductive phase transition share some major regulatory factors. In addition, many of these factors also affect some of the heteroblastic features that distinguish these two phases. In particular, two evolutionary highly conserved microRNAs (miRNAs), miR156 and miR172, and their targets have been identified as key components of the genetic control mechanisms that underlie plant phase changes. The miRNA miR156 targets transcripts of a subset of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) transcription factors. These were originally identified in inflorescence nuclear protein

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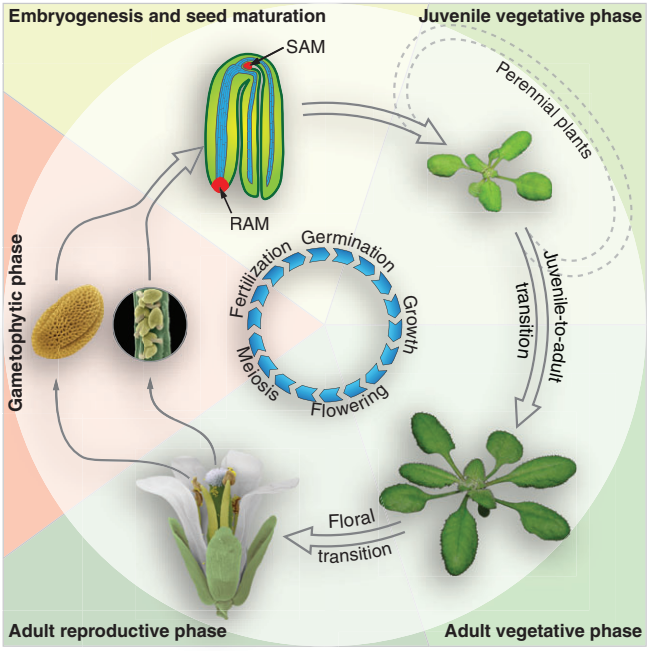


Fig. 1. Phases of plant development. Plants progress through a number of developmental transitions during their life cycle. During sexual reproduction, gametes are produced (gametophytic phase). After fertilization, populations of stem cells are established at the opposing ends of the primary growth axis of the developing embryo, forming the root apical meristem (RAM) and the shoot apical meristem (SAM) (red). These meristems give rise to all post-embryonic organs formed throughout the life of a plant. The entire aerial part (shoot) of a plant originates from the SAM. After germination, plants generally pass through three more-or-less discrete developmental phases. First, the shoot passes through a phase of vegetative growth during which the plant rapidly increases in size and mass. The vegetative growth phase can be further divided into juvenile and adult phases of vegetative development. Eventually, plants become competent to flower and undergo the transition to reproductive development. During this phase, the SAM gives rise to flowers instead of shoots. Within the flower, male and female gametes form, which enter short gametophytic (haploid) phases of development before fusing to form a new diploid zygote (start of sporophytic phase).

extracts by means of their conserved DNA-binding SBP domain (Klein et al., 1996) and more recently have been shown to promote the transition from juvenile to adult and to flowering (Schwarz et al., 2008; Wu and Poethig, 2006). By contrast, miR172 targets mRNAs that encode proteins with two APETALA 2 (AP2) DNA-binding domains. These proteins have been shown to regulate both the transition to flowering and flower development (Aukerman and Sakai, 2003; Jung et al., 2007; Mathieu et al., 2009; Schmid et al., 2003). Furthermore, the MADS-domain transcription factor FLOWERING LOCUS C (FLC), a key player in the *Arabidopsis* vernalization response (Amasino, 2010; Srikanth and Schmid, 2011), has recently been shown to delay the juvenile-to-adult transition by directly acting on some of the same targets (Willmann and Poethig, 2011; Deng et al., 2011). These findings further demonstrate that tight connections exist between the juvenile-to-adult and the reproductive phase transitions.

In this review, we discuss recent findings, mainly from *Arabidopsis*, that have increased our understanding of the regulation of phase transitions in plants. In particular, we focus on the roles of miR156 and miR172, and their targets, in these processes. We further highlight a possible reinforcing feedback loop between these miRNAs and their targets, and discuss the potential role of this regulatory feedback loop in establishing a sharp boundary between different floral organs. Finally, we review the roles of these miRNAs in other plant species, such as maize and poplar, and discuss the evolutionary conservation of the function of these miRNAs.

Plant phase changes and heteroblasty

As a plant passes through the various developmental phases of its life cycle and continues to develop new organs, a number of morphological traits (such as size and shape of leaves, phyllotaxy, plastochron, internode length, adventitious root production, trichome distribution and cell size) change in accordance with the developmental stage. As a result, different parts of a plant may exist in different developmental phases. This phenomenon of heteroblasty is observed in many species. It should be noted, however, that morphological plasticity, expressed as a consequence of environmental variations, often affects the expression of the very same heteroblastic features. It can, thus, be difficult to distinguish genetically determined ontogenetic changes from those that occur due to plasticity (Diggle, 2002). Like the acquisition of reproductive competence (Mozley and Thomas, 1995), most of the heteroblastic features change gradually. These features can be classified as either juvenile or adult, and are often used as markers to monitor progression of the juvenile-to-adult phase change. Although these changes are collectively known as the vegetative phase change (see Glossary, Box 1), it is important to note that it is not yet clear how the phenomenon of heteroblasty relates to the reproductive competence of the shoot. In addition, the use of the terms ‘juvenile’ and ‘adult’ in describing both the heteroblastic change as well as the state of reproductive competence, may lead to confusion, as discussed by Jones (Jones, 1999) and others [Zotz et al. (Zotz et al., 2011) and references to older literature on heteroblasty therein].

Despite the appearance of similar heteroblastic traits that often accompany particular developmental phase changes in different plant species, the necessity or consequences of these relationships, from a plant-fitness perspective, remain poorly understood. Elucidating the molecular genetic mechanisms that link these phenomena is, thus, of immediate importance if we are to achieve a better understanding of plant diversification and distribution.

Large differences exist among species with regards to the extent to which particular heteroblastic changes are expressed. They are usually most apparent in perennial woody plants, in which individual developmental phases can last up to several years. Moreover, branching, in combination with seasonality in vegetative and reproductive growth, allows perennial woody plants to bear shoots in different, i.e. juvenile or adult, developmental stages, making them optimal systems for phenotypic studies of vegetative phase change (Doorenbos, 1965; Hackett, 1985; Jaya et al., 2010). Unfortunately, such plants are usually not the best suited for molecular analyses as the genomic resources available for them are still limited, and experiments with these large and slow-growing species are often space demanding and time consuming. However, building on observations made in *Arabidopsis*, some remarkable progress in this area has recently been achieved in poplar (Hsu et al., 2011; Wang et al., 2011).

Fortunately, vegetative phase change, albeit less dramatic, can also be observed and studied in small annual plants, such as in the model plant *Arabidopsis thaliana* (Steynen et al., 2001; Telfer et al., 1997; Tsukaya et al., 2000; Martinez-Zapater et al., 1995; Röbbelen, 1957).

Box 1. Glossary

Adult phase. A later phase of growth following a juvenile growth phase that can be subdivided into an adult vegetative phase, during which the ability to reproduce sexually under normal conditions is acquired, and a subsequent adult reproductive phase, which is characterized by the actual realization of reproductive structures.

Callus. A mass of proliferating undifferentiated cells that is induced by wounding or by the application of plant hormones to an in vitro culture of differentiated plant tissues.

Floral meristem. Determinate meristem that produces a defined set of specialized perianth and reproductive organs, and that terminates with the production of carpels.

Floral transition. More correctly known as the 'transition to flowering'. It is interchangeable with the concept of 'reproductive phase transition' (see definition below).

Heteroblasty. The anatomical and morphological differences in plant (shoot) organs produced in the earlier (juvenile) and later (adult) stages of development. It reflects specific ontogenetic changes and not phenotypic plasticity in response to ambient conditions.

Inflorescence. After the reproductive phase/flowering transition, the shoot apical meristem ceases to produce vegetative leafy shoots and becomes an inflorescence meristem (IM), producing flowers or flowering shoots instead. An IM is either indeterminate (when it reiteratively forms lateral inflorescence or floral meristems) or determinate (when it self-converts into a floral meristem).

Juvenile phase. Phase of vegetative growth, generally following germination, during which the plant (shoot) forms true leaves and axillary buds but is incapable of sexual reproduction, i.e. it is not flowering competent.

Juvenile-to-adult phase transition. Change from the juvenile to the adult phase of vegetative shoot development, when the plant acquires reproductive competence.

Meristem identity transition. The SAM can adopt different identities, e.g. vegetative, inflorescence or floral (see above). During the transition from one identity to the next, meristem features (such as lateral organ type, phyllotaxy, internode elongation and indeterminate growth) that were characteristic of the previous phase become replaced by those that are characteristic of the next phase.

Plastochron. The time interval between initiation of successive leaf or flower primordia at the shoot apical meristem.

Reproductive phase transition. The transition from vegetative to reproductive growth when the competent SAM responds to signals that evoke floral initiation and, after evocation, becomes determined to flower. It generally follows the vegetative phase transition but as both can be under different controls, they can be uncoupled (as in many woody species).

Shoot apical meristem. The SAM harbours a population of slowly proliferating stem cells whose descendants give rise to all aerial parts of a plant. The SAM allows the plant to generate new organs during its entire life, and underlies the possible existence of shoots in different phases of maturity, e.g. juvenile or adult, on the same plant.

Vegetative phase change. The change between a morphological distinct early phase, i.e. with 'juvenile' features, and a late phase, i.e. with 'adult' features, during vegetative plant (shoot) development. It need not necessarily be coupled to or reflect a change in reproductive competence.

Vernalization. Exposure to a prolonged period of cold, e.g. winter, required by many plant species to break seed or bud dormancy and to facilitate flowering.

The vegetative phase of shoot development in *Arabidopsis* lasts between weeks in summer-annual varieties and up to several months in winter-annual accessions that require vernalization to induce flowering (Amasino, 2010). As part of the vegetative phase transition, the juvenile-to-adult phase transition in *Arabidopsis* is accompanied by relatively minor changes in leaf morphology (Chien and Sussex, 1996; Telfer et al., 1997; Telfer and Poethig, 1994; Usami et al., 2009). The early rosette leaves that are produced feature 'juvenile traits', including long petioles, small almost round blades and smooth margins. In addition, juvenile leaves produce trichomes on only their upper (adaxial) side. Late rosette leaves, by contrast, display 'adult traits', such as shorter petioles, enlarged and elongated blades with an increased number of size-reduced cells, as well as serrated margins and trichomes on both their upper and lower (abaxial) side. With the latter as a notable exception, these features change gradually, and the so-called transition leaves may display a mix of juvenile and adult features. As the absence or presence of abaxial trichomes is easy to score, and because their production is affected by photoperiodic conditions and by mutations that affect flowering time (Telfer et al., 1997), this feature is frequently used to quantify the duration of the juvenile phase (e.g. the duration of the juvenile phase can be expressed as the number of rosette leaves without abaxial trichomes). As such, a number of recent insights into the genetic control of phase changes have been gained from studying *Arabidopsis*.

Control of phase transitions by miRNAs and their targets

As mentioned above, both intrinsic and environmental conditions can affect heteroblastic features. For example, lengthening or shortening the daily light period affects the number of nodes that bear 'juvenile' leaves (Chien and Sussex, 1996; Telfer et al., 1997). The importance of different regulatory systems can thus be revealed in specific mutants. For example, *Arabidopsis* mutants that are insensitive to the phytohormone gibberellin (GA) or those defective in its biosynthesis show a delayed appearance of the first adult leaf (Chien and Sussex, 1996; Telfer et al., 1997). Interestingly, another class of mutants that display accelerated vegetative phase changes are those associated with mutations in genes involved in the biogenesis and action of miRNAs and of trans-acting small interfering RNAs (ta-siRNAs). Whereas the effects of ta-siRNAs on heteroblasty may be limited to their ability to affect leaf polarity via their targeting of auxin response factors (Allen and Howell, 2010; Fahlgren et al., 2006; Hunter et al., 2006; Pekker et al., 2005), the effects of disturbed miRNA biogenesis are more diverse. The pleiotropic phenotypes associated with perturbed miRNA function are perfectly understandable in the light of the plethora of currently known plant miRNAs (Rubio-Somoza and Weigel, 2011). However, when the first miRNAs were identified in plants, it was soon recognized that many of the predicted targets encode transcription factors, some of which were known to affect flowering (Rhoades et al., 2002). Further insights into the role of individual miRNAs and their transcription factor targets in developmental phase changes have since been gained from misexpression studies in *Arabidopsis*.

The miR156/SPL regulatory module in *Arabidopsis* phase transitions

miR156 promotes the juvenile phase

One of the most abundant miRNAs in *Arabidopsis* is miR156, which reaches it highest levels at the seedling stage (Axtell and Bartel, 2005; Fahlgren et al., 2007; Wang et al., 2009). When constitutively overexpressed, miR156 causes a moderate delay in flowering (Fig. 2) (Schwab et al., 2005). Moreover, such transgenics produce a larger number of leaves with characteristically juvenile features (Wu and Poethig, 2006).

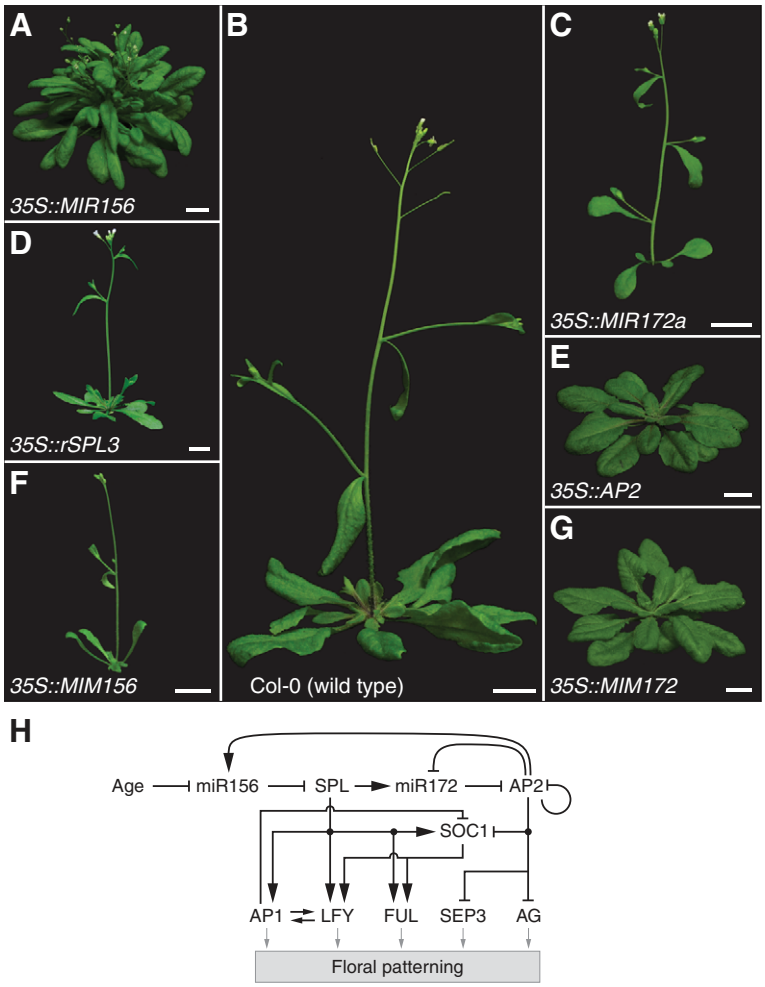


Fig. 2. Opposing effects of miR156, miR172 and their respective targets on *Arabidopsis* development. (**A**) Constitutive overexpression of *MIR156* results in prolonged vegetative phase (e.g. an increased number of juvenile leaves) and reduced apical dominance, i.e. the simultaneous bolting of multiple shoots when compared with (**B**) Col-0 (wild type) control. (**C**) A plant constitutively overexpressing *MIR172*, which, by contrast, essentially skips the juvenile vegetative growth phase and flowers after producing only two or three small adult leaves. (**D**) A plant constitutively overexpressing a miRNA-resistant form of the miR156 target *SPL3*, which reduces the duration of the juvenile phase and promotes flowering. (**E**) A plant constitutively overexpressing the miR172 target *AP2*, which results in late flowering. (**F**,**G**) The effects of miRNA target overexpression can be recapitulated by constitutively expressing the target mimics *MIM156* and *MIM172*, which reduce the functional levels of the mature miR156 and miR172 miRNAs, respectively. All plants were grown under long day photoperiod (16 hours of light, 8 hours of darkness). Scale bars: 1 cm. (**H**) A model of the sequential action of miR156, miR172 and their respective targets in regulating phase transitions in *A. thaliana*. Abbreviations: 35S, cauliflower mosaic virus promoter; AG, AGAMOUS; AP1, APETALA1; AP2, APETALA2; FUL, FRUITFULL; LFY, LEAFY; MIM156, miR156 mimicry target; MIM172, miR172 mimicry target; miR156, mature miRNA156; miR172, mature miRNA172; MIR156, miRNA156 gene; MIR172, miRNA172 gene; rSPL3, miR156-resistant form of SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3; SEP3, SEPALLATA3; SOC1, SUPRESSOR OF OVEREXPRESSION OF CONSTANS 1; SPL, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE.

Elevated levels of miR156 thus seem to prolong juvenility and delay the onset of the adult phase. In *Arabidopsis*, miR156 and some miR156 isoforms can potentially be encoded by many loci, e.g. *MIR156a-f*, *MIR156g-h* and *MIR157a-d* (Kozomara and Griffiths-Jones, 2011; Xie et al., 2005). The sequestration of miR156 upon the overexpression of miR156 mimicry targets (*MIM156*) has been used to perturb endogenous miR156 function. Such *MIM156* transgenics indeed flower after producing only a few leaves, which all display adult features (Fig. 2) (Franco-Zorrilla et al., 2007; Todesco et al., 2010). miR156 is thus not only sufficient but also necessary for the expression of the juvenile phase. miR156 levels are highest in *Arabidopsis* seedlings and, in full agreement with its role in regulating the juvenile-to-adult phase transition, these levels decline further during development (Wang et al., 2009; Wu and Poethig, 2006). How this decline is accomplished remains unknown. In seedlings, miR156 levels remain largely unaffected in both loss- and gain-of-function studies of the core factors that act in the well-established flower-promoting pathways (Wang et al., 2009). Low temperatures may have a mild positive effect on a few *MIR156* loci but none of the known ambient temperature pathway components (Lee et al., 2007) seems to affect miR156 accumulation (Lee et al., 2010). miR156 thus seems to define an independent endogenous flowering pathway that acts in an age-dependent manner. The nature of the age-dependent factor(s) that regulate the *MIR156* loci remains a mystery but recent organ ablation experiments suggest that leaves act as a source of a miR156-repressing factor (Yang et al., 2011).

SPL genes promote the adult phase

Whereas the molecular genetic factors that act immediately upstream of *MIR156* remain to be discovered, the events downstream of *MIR156* expression have already been unveiled. In *Arabidopsis*, the targets of miR156 include transcripts of 11 of the 17 SPL genes, and other miR156 targets that represent orthologues or likely orthologues of SPL-like genes have been identified in other species (summarized in Table 1). Experimental evidence that the miRNA recognition elements (MREs) of these transcripts are indeed responsive and functional is diverse and convincing (Franco-Zorrilla et al., 2007; Martin et al., 2010b; Park et al., 2005; Schwab et al., 2005; Shikata et al., 2009; Usami et al., 2009; Wang et al., 2009; Wang et al., 2008; Wu et al., 2009; Wu and Poethig, 2006; Xing et al., 2010).

As unique targets of miR156, SPL genes are expected to play key roles in the vegetative and possibly the reproductive phase change, but, probably owing to extensive redundancy, single mutants of SPL genes do not show strong phenotypes. The analysis of specific targets or groups of targets informed by phylogeny and, in particular, paralogous relationships (Guo et al., 2008; Riese et al., 2007; Xie et al., 2006), has helped to unveil their respective roles in developmental phase transitions.

The closely related genes *SPL3*, *SPL4* and *SPL5*

The three smallest of all SPL genes in *Arabidopsis*, *SPL3*, *SPL4* and *SPL5* are exceptional in that they carry a miR156 MRE in their 3′ UTRs (Gandikota et al., 2007). They are orthologous to the very

Table 1. miRNA156 genes and targets

Gene	Species	Locus identifier*	Function/comments	References
MIR156	*A. thaliana*	a) At2g25095 b) At4g30972 c) At4g31877 d) At5g10945 e) At5g11977 f) At5g26147 g) At2g19245 h) At5g55835	Promotes juvenile phase; targets 11 out of 17 SPL transcripts in *A. thaliana*	Axtell and Bowman, 2008 Cuperus et al., 2011 Lee et al., 2010 Rhoades et al., 2002 Schwab et al., 2005 Wang et al., 2009 Wu et al., 2009 Wu and Poethig, 2006 Xie et al., 2005 Yang et al., 2011
Corngrass1 (*Cg1*)	*Z. mays*		Promotes juvenile phase; tandem duplication of miR156 genes (*zma-miR156b* and *zma-miR156c*)	Chuck et al., 2007a
SPL2	*A. thaliana*	At5g43270	Regulator of leaf shape	Nodine and Bartel, 2010 Shikata et al., 2009
SPL3	*A. thaliana*	At2g33810	Promoter of flowering	Cardon et al., 1997 Gandikota et al., 2007 Wang et al., 2009 Wu et al., 2009 Yamaguchi et al., 2009
PcSPL3	*P. x canadensis*	XM_002329758	Locus-id refers to homologous gene of *P. trichocarpa*	Wang et al., 2011
SBP1	*A. majus*	X92369.1	Promoter of flowering; a likely *SPL3* ortholog; founding member of the SBP-box gene family	Klein et al., 1996 Preston and Hileman, 2010
CNR	*S. lycopersicon*	SGN-U317177	Promoter of fruit ripening; a likely *SPL3* ortholog	Manning et al., 2006 Moxon et al., 2008 Zhang et al., 2011
SPL4	*A. thaliana*	At1g53160	Promoter of flowering	Gandikota et al., 2007 Wu et al., 2009 Wu and Poethig, 2006
SPL5	*A. thaliana*	At3g15270	Promoter of flowering	Gandikota et al., 2007 Wu et al., 2009 Wu and Poethig, 2006
SPL9	*A. thaliana*	At2g42200	Promotes juvenile-to-adult phase transition and flowering; directly regulates genes involved in trichome formation	Schwarz et al., 2008 Wang et al., 2009 Wang et al., 2008 Wu et al., 2009 Yu et al., 2010
PcSPL9	*P. x canadensis*	XM_002322642.1	Locus-Id refers to homologous gene of *P. trichocarpa*	Wang et al., 2011
OsSPL14	*O. sativa*	Os08g39890	Promoter of grain yield; a likely *SPL9* orthologue	Jiao et al., 2010 Miura et al., 2010 Xie et al., 2006
SPL10	*A. thaliana*	At1g27370	Regulator of leaf shape and epidermal traits	Nodine and Bartel, 2010 Shikata et al., 2009 Wu et al., 2009
SPL11	*A. thaliana*	At1g27360	Regulator of leaf shape; a *SPL10* paralogue	Nodine and Bartel, 2010 Shikata et al., 2009
SPL13	*A. thaliana*	a) At5g50570 b) At5g50670	Regulates the switch from cotyledon to vegetative leaf stage	Martin et al., 2010a Martin et al., 2010b
SPL15	*A. thaliana*	At3g57920	Acts redundantly with its likely paralogue *SPL9*	Schwarz et al., 2008 Usami et al., 2009 Wang et al., 2008
tasselsheath4 (*tsh4*; *ZmSBP6*)	*Z. mays*		SBP-box gene most similar to *Arabidopsis SPL9* and *SPL15*; involved in bract development	Bensen et al., 1995 Chuck et al., 2010 Hultquist and Dorweiler, 2008
teosinte glume architecture 1 (*tga1*)	*Z. mays*		SBP-box gene most similar to *Arabidopsis SPL13*; involved in ear glume development; controlled a key event in the domestication of maize	Hultquist and Dorweiler, 2008 Wang et al., 2005

*Gene identifier under which it can be found in public databases.

Abbreviations: *CNR*, *COLORLESS NONRIPENING*; *Os, O. sativa; Pc*, *P. x canadensis*; *SBP*, *SQUAMOSA PROMOTER-BINDING PROTEIN*; *SPL*, *SQUAMOSA PROMOTER-BINDING PROTEIN LIKE*.

first SBP-box genes identified in snapdragon, which were originally proposed to regulate flowering (Klein et al., 1996). In *Arabidopsis,* constitutive overexpression of a miR156-resistant *SPL3* (*rSPL3*) transgene, indeed results in early flowering (Fig. 2D) (Cardon et al., 1997), and similar results can be obtained for *rSPL4* and *rSPL5* (Wu and Poethig, 2006). Moreover, such transgenics show a precocious appearance of adult leaf traits, providing clear support that these SPL genes promote the expression of the adult phase (Gandikota et al., 2007; Wang et al., 2009; Wu et al., 2009; Wu and Poethig, 2006). Transgenics that constitutively overexpress *SPL3* flower only slightly earlier than do wild-type plants, reflecting the repressing strength of endogenous miR156 (Gandikota et al., 2007; Wu and Poethig, 2006).

The closely related genes *SPL9* and *SPL15*

Single loss-of-function mutants for *SPL9* or *SPL15* do not show strong phenotypes (Schwarz et al., 2008). However, a double mutant for these likely paralogous genes mimics mild miR156 overexpressors, indicating a role for these genes in promoting the juvenile-to-adult phase transition (Schwarz et al., 2008; Wang et al., 2008). In fact, the expression of a miR156-insensitive form of *SPL9* (*rSPL9*) results in plants that virtually skip the juvenile phase (Wang et al., 2009; Wu et al., 2009). Furthermore, *SPL9*, through miR172, promotes the characteristic appearance of abaxial trichomes on adult leaves, possibly through the direct transcriptional activation of *MIR172b* (Wu et al., 2009). In turn, miR172 promotes abaxial trichome production at least in part by repressing *TARGET OF EAT1* (*TOE1*) and *TOE2* (discussed in more detail later). *SPL9* also exerts control over the acropetal change in trichome density along the shoot, another hallmark of vegetative phase change (Telfer et al., 1997), by directly regulating two negative regulators of trichome formation: *TRICHOMELESS1* and *TRIPTYCHON* (Yu et al., 2010).

SPL9 and *SPL15* control not only phase dimorphisms, but also the competence to flower in response to the appropriate photoperiod (Schwarz et al., 2008). Furthermore, an *spl9 spl15* double mutant partly phenocopies transgenics that constitutively overexpress miR156, such that they both exhibit shortened plastochron (Schwarz et al., 2008; Wang et al., 2008). Interestingly, Wang et al. (Wang et al., 2008) could detect *SPL9* expression in leaf anlagen and primordia but not in the shoot apical meristem (SAM). In addition, the authors observed that elevated expression of *rSPL9* under leaf primordia-specific promoters increased plastochron (see Glossary, Box 1) length, and concluded that the effect of *SPL9* on the initiation of new leaf primordia is due to its expression in existing leaf primordia. The nature of this signalling remains unknown but may be related to cell proliferation and expansion, features affected by the *more and smaller cells* (*msc*) mutants, of which one (the *msc1* mutant) represents a miR156-resistant allele of *SPL15* (Usami et al., 2009).

SPL9 and *SPL15* seem to share functional redundancy with *SPL3/4/5* with respect to their effects on abaxial trichome production and petiole length, but not with regard to their effects on leaf shape (Wu et al., 2009) or plastochron length (Wang et al., 2008). Some functional specialization with respect to different features of shoot development may thus already be recognizable between these subgroups of miR156-targeted SPL genes.

The closely related genes *SPL2*, *SPL10* and *SPL11*

Loss of *SPL2* function weakly enhances the *spl9 spl15* double mutant phenotype (Schwarz et al., 2008). Phylogenetic analysis places *SPL2* in the same clade as *SPL10* and *SPL11* (Riese et al., 2007). Single mutants, as well as the double *spl2 spl10* and *spl2 spl11* mutants, look similar to wild type (Shikata et al., 2009; Wu et al., 2009). The *SPL10* and *SPL11* loci are in close physical proximity, complicating the generation of triple mutants to study further redundancy. Shikata et al. (Shikata et al., 2009) circumvented this problem by generating plants that express dominant repressor versions of the respective genes. In these transgenics, the rosette leaves were narrower but abaxial trichome initiation and flowering time remained unaffected. However, the expression of miR156-insensitive versions of *SPL10* and *SPL11* (*rSPL10* and *rSPL11*) accelerated the expression of adult traits, producing phenotypes that are similar, but not identical, to those manifested by *rSPL9* (Wu et al., 2009). In normal development, *SPL2*, *SPL10* and *SPL11* thus only weakly affect leaf shape, indicating that they have a minor role in the vegetative phase change. Actually, this subgroup of miR156-targeted SPL genes may play its major role in early embryonic patterning by redundantly regulating the embryonic morphogenesis-to-maturation phase transition (Nodine and Bartel, 2010). Within this context, it is interesting to note that Luo et al. (Luo et al., 2006) observed a striking increase in miR156 levels associated with differentiating rice embryonic calli (see Glossary, Box 1) in vitro, suggesting a role for miR156, and thereby for its targets, in embryogenesis.

The remaining miR156-targeted genes *SPL6* and *SPL13*

Our current knowledge on the roles of the remaining miR156-targeted SPL genes, *SPL6* and *SPL13*, is still very limited. To date, loss- or gain-of-function *SPL6* mutant phenotypes have not been reported. *SPL13*, however, may play a role in the cotyledon to vegetative-leaf stage switch, as the expression of a miR156-resistant *SPL13* transgene (*rSPL13*) results in a slight delay in the emergence of the first true leaves, an effect mediated by the miR172 target *SCHNARCHZAPFEN* (*SNZ*) (Martin et al., 2010a; Martin et al., 2010b).

miR156-targeted SPL genes also promote the transition to flowering

As direct targets of miR156, genes such as *SPL3*, *SPL4* and *SPL5*, as well as *SPL9* and *SPL15*, have been shown to be important determinants in both the vegetative phase transition and flowering competence. Accordingly, and concomitantly with decreasing miR156 activity, their transcript levels gradually increase during aging (Cardon et al., 1999; Schmid et al., 2005; Wu and Poethig, 2006).

As mentioned before, vernalization, photoperiod and GA-dependent flowering pathways do not have an obvious effect on miR156 levels in seedlings. In addition, these flowering pathways also leave the expression levels of miR156 targets such as *SPL3* and *SPL9* largely unaffected in seedlings (Wang et al., 2009). Upon photoperiodic induction of adult plants, however, *SPL3*, *SPL9* and other miR156-targeted SPL genes become strongly upregulated in the shoot apex (Schmid et al., 2003), indicating an additional role in establishing inflorescence meristem or floral meristem (see Glossary, Box 1) identity.

One of the major events in photoperiodic induction in *Arabidopsis*, i.e. the generation of a flower-promoting and leaf-derived signal that is sensed by the SAM, involves the activation of *FLOWERING LOCUS T* (*FT*) in the leaf. FT protein is subsequently translocated to the shoot apex where its interaction with the bZIP transcription factor FD results in the activation of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and a meristem identity switch from vegetative into inflorescence. Together, FT/FD and SOC1 are able to activate *LEAFY* (*LFY*), *APETALA1* (*AP1*) and

FRUITFULL (*FUL*), which subsequently show mutual activation but negatively feed back on *SOC1*, thereby promoting the switch from IM to FM identity (Amasino, 2010; Srikanth and Schmid, 2011). The *Arabidopsis* inflorescence, however, remains indeterminate as *TERMINAL FLOWER1* prevents the activation of these floral meristem identity genes in the IM itself such that only its lateral meristems become transformed into flowers (Preston, 2010).

Different lines of evidence show that *SPL3* and *SPL9*, despite being from different SPL clades, target the same floral meristem identity genes. Using X-ChIP, Yamaguchi et al. (Yamaguchi et al., 2009) showed that SPL3 can bind to promoter and intragenic regions of *LFY*, *AP1* and *FUL* in vivo. In addition, Wang et al. (Wang et al., 2009) reported SPL9 to be a direct regulator of *FUL* and *AP1*, and identified *SOC1* and its likely paralogue *AGL42* as further direct targets.

In order to promote flowering, a photoperiod-dependent pathway and an age-dependent pathway (i.e. a pathway activated by the seemingly autonomous decline in miR156 activity), thus converge through FT/FD and miR156-targeted SPL genes, respectively, on a set of target genes that determine inflorescence and floral meristem identity. The absence of SPL activity, as a consequence of high miR156 levels early in shoot development, renders the plant insensitive to FT/FD-dependent flower induction. However, the ability of these photoperiod-dependent factors to induce flowering becomes established when miR156 levels decline and the expression of its targets increases. Notably, when miR156-targeted SPL activity continues to rise, plants will eventually flower without the requirement for photoperiod-dependent FT/FD activity (Wang et al., 2009). In fact, overexpression of a *rSPL3* transgene may result in a complete consumption of the IM in creating floral organs and thereby in determinate growth of the inflorescence (Gandikota et al., 2007).

miR156-targeted SPL genes promote fertility

The first few flowers formed in *Arabidopsis* often produce significantly fewer seeds than do later flowers, which may be due to an inflorescence architectural effect, i.e. dependent on the position of the flower in the inflorescence and not only to environmental variation (Diggle, 2002). Interestingly, miR156-targeted SPL genes function redundantly with the *SPL8* gene, which is not targeted by miR156, in promoting *Arabidopsis* flower fertility (Xing et al., 2010). Furthermore, miR156-targeted SPL gene activity has recently been coupled to increasing flavonol to anthocyanin ratios within the developing *Arabidopsis* shoot (Gou et al., 2011). Anthocyanins, like trichomes, may discourage small herbivores at early stages of development, whereas flavonoids are required for full fertility at later stages of development (Thompson et al., 2010). An increase in miR156-targeted SPL gene activity even after plants have bolted and initiated their first flowers may thus explain the ontogenetic shift observed in the degree of flower fertility and seed set.

Phase transition control by miR172 and AP2-like transcription factors

miR172/AP2 modulate vegetative phase change in *Arabidopsis*

In addition to miR156, the miRNA miR172 and its targets have been implicated in phase transitions in *Arabidopsis*. miR172 levels increase as plants age, in a pattern that is complementary to that of miR156 (Fig. 3). In contrast to miR156, the abundance of which is controlled by plant age, miR172 expression appears to be under photoperiodic control (Jung et al., 2007). miR172 has been found to target several transcripts that encode transcriptions factors involved in the repression of flowering (Table 2). For example, the transcripts of six genes in *Arabidopsis* that all encode AP2-type transcription factors are targeted by miR172 (Chen, 2004). One of these is *AP2* itself, a gene well known for its role in floral patterning (Bowman et al., 1991; Jofuku et al., 1994). The other five targets, *TOE1*, *TOE2*, *TOE3*, *SCHLAFMÜTZE* (*SMZ*) and *SNZ*, also act as repressors of flowering (Aukerman and Sakai, 2003; Jung et al., 2007; Mathieu et al., 2009; Schmid et al., 2003). However, only recently has it been shown that miR172 and its targets also control the juvenile-to-adult transition in *Arabidopsis*. In particular, the overexpression of miR172 and its targets (Aukerman and Sakai, 2003; Chen, 2004; Jung et al., 2007; Mathieu et al., 2009; Schmid et al., 2003) results in effects that are opposite to those caused by miR156 overexpression (Fig. 2C,E,G). Wu et al. (Wu et al., 2009) also reported that in transgenic plants that constitutively overexpress miR172, abaxial trichomes are produced earlier than is normal. Conversely, the formation of abaxial trichomes is delayed in the *mir172a* mutant. In either case, leaf shape is morphologically normal, indicating that MIR172 genes affect only a subset of the traits (e.g. epidermal patterning) that are normally associated with juvenility.

MIR172b is a direct transcriptional target of SPL9, as demonstrated by chromatin immunoprecipitation and by the induction of miR172 in transgenics in which SPL9 activity can be conditionally induced (Wu et al., 2009). This finding suggested that SPL proteins might promote adult epidermal identity by indirectly repressing miR172-targeted AP2-like transcription factors. Indeed, further analyses revealed that the loss of *SPL9* delayed the appearance of abaxial trichomes in a *toe1 toe2* double mutant. However, the observed effect was rather mild, indicating that *TOE1* and *TOE2* only contribute to, but are not solely responsible for, this epidermal phenotype (Wu et al., 2009).

In summary, it appears that, via their antagonistic function, miR156 and miR172 ratios determine the timing of the juvenile-to-adult transition, and that the vegetative phase change in particular is controlled by their sequential activity (Fig. 3).

miR172/AP2 regulate transition to flowering

In addition to their rather subtle role in promoting juvenile epidermal traits, miR172 and its targets have an additional function in regulating the transition to flowering. This was first made apparent when Aukerman and Sakai (Aukerman and Sakai, 2003) isolated an extremely early flowering mutant from an activation-tagging screen and identified *MIR172b* as the causal gene. The same screen also identified the late flowering *toe1-D* mutant, which was found to overexpress the miR172 target *TOE1*. Analyses of loss-of-function alleles revealed that *toe1* mutants flowered significantly earlier than did wild-type plants, and that this phenotype was enhanced in a *toe1 toe2* double mutant that also lacked the closely related *TOE2* gene (Aukerman and Sakai, 2003; Jung et al., 2007). However, even the double mutant flowered significantly later than miR172b-overexpressing lines, suggesting that other factors act redundantly with TOE1 and TOE2 to repress flowering.

Schmid and colleagues (Schmid et al., 2003) reported that overexpression of *SMZ* also significantly delayed flowering, particularly under the condition of long days. In contrast to *toe1*, plants mutant for *SMZ* or for its paralog *SNZ*, did not flower earlier than control plants. Loss of either *SMZ* or *SNZ* function, however, did further accelerate flowering when combined with *toe1* and *toe2*. Nevertheless, even in the quadruple *toe1 toe2 smz* and *snz* mutant, flowering occurred significantly later than in transgenics constitutively overexpressing miR172 (Mathieu et al., 2009), suggesting further redundancy within this gene family. This was later confirmed when it was found that AP2, besides its well-

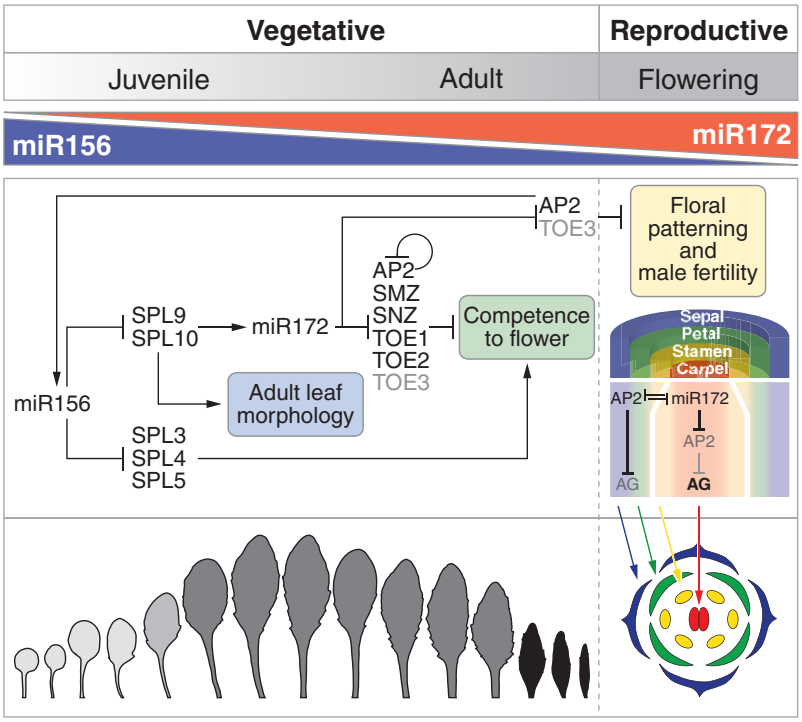


Fig. 3. Regulation of phase change in *Arabidopsis*. During early development, the levels of miR156 are initially high, promoting the juvenile vegetative growth phase in seedlings. Juvenile leaves (light grey, lower left) are almost round in shape and exhibit trichomes only on their adaxial side. As the plant matures, the levels of miR156 steadily decrease, allowing for the production of SPL9 and SPL10 proteins that promote adult leaf traits (dark grey; elongated leaves with abaxial trichomes). At the same time, SPL9 and SPL10 directly induce the expression of *MIR172* genes. Increased levels of miR172 result in the downregulation of six AP2-like transcription factors that normally repress flowering. Release from this repression, in combination with the flower-promoting actions of SPL3, SPL4 and SPL5, makes the plant competent to flower and the transition to flowering can occur. During the transition to flowering, the shoot apical meristem does not immediately give rise to flowers but rather to secondary shoots that emerge from the axils of cauline leaves (black, lower right). In addition to its role as a floral repressor (lower right; from above, bottom; in longitudinal section, above), AP2 contributes to the patterning of the emerging flower. Both AP2 and miR172 participate in establishing a sharp boundary between the vegetative outer organs (sepals, petals) and the inner whorls of reproductive organs (stamen, carpels). Abbreviations: AG, AGAMOUS; AP2, APETALA2; miR156, mature miRNA156; miR172, mature miRNA172; SMZ, SCHLAFMÜTZE; SNZ, SCHNARCHZAPFEN; SPL, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE; TOE, TARGET OF EARLY ACTIVATION TAGGED.

described role in floral patterning, also functions as a floral repressor (Yant et al., 2010). Eventually, a hextuple mutant lacking the functions of all six miR172 targets was found to essentially phenocopy the effect of constitutive miR172 expression (Yant et al., 2010).

The transition to flowering is under the control of a complex genetic network that perceives and integrates various endogenous and environmental cues. Boss and colleagues (Boss et al., 2004) have proposed that the different pathways that regulate flowering can be viewed as either pathways that actively promote flowering, such as the photoperiod, light quality, gibberellic acid and ambient temperature pathways, or pathways that enable flowering by removing floral repressors from the system. According to this hypothesis, miR156-targeted SPL genes and miR172 can be considered 'enabling factors', as their activity ultimately leads to the shutting down of the AP2-like floral repressors that regulate 'meristem competence' (Bernier, 1988). In this light, miR156, which has been shown to prevent precocious flowering (Wang et al., 2009) until plants have reached a permissive age, can be considered as an 'enabling factor'. However, the miR156-targeted SPL genes do not only regulate the expression of miR172 and thus the AP2-like floral repressors, SPL proteins have also been shown to directly bind to and promote the expression of floral integrator genes, such as *SOC1* (Wang et al., 2009) and floral meristem identity genes such as *LFY*, *FUL* and *AP1* (Yamaguchi et al., 2009). As often occurs, the more we learn, the more complex the situation becomes.

Regulation of flower development by miR172/AP2

Besides its role in regulating phase transitions, miR172 and its targets appear to also play a role in floral patterning. The angiosperm prototype flower consists of four different organs that are organized in concentric whorls. The patterning of the flower is explained by the 'classical' ABC model (see Box 2), according to which the combinatorial interaction of three classes of homeotic functions (A, B and C), provides the positional information that determines the fate of the emerging floral organs (Causier et al., 2010; Lohmann and Weigel, 2002). In brief, in the outermost whorl of the *Arabidopsis* flower, sepals are specified by the activity of AP1 and AP2 (A class). In the second whorl, the activity of A-class proteins overlaps with those of the B-class proteins PISTILLATA and APETALA3 to establish petals. Similarly, overlapping activity of the B-class proteins with that of the C-class protein AGAMOUS (AG) induces stamen fate in the third whorl. Finally, in the innermost whorl, AG controls the formation of carpels. An important feature of the ABC model is that A-class and C-class function are mutually exclusive. More recently, four highly redundant

Table 2. miR172: its genes and targets

Gene	Species	Locus identifier*	Function/comments	References
MIR172	*A. thaliana*	a) At2g28056 b) At5g04275 c) At3g11435 d) At3g55512 e) At5g59505	Promotes adult vegetative phase and flowering; involved in floral patterning; targets six AP2-like transcripts in *A. thaliana*; *MIR172b=EARLY ACTIVATION TAGGED* (*EAT*)	Aukerman and Sakai, 2003 Axtell and Bowman, 2008 Cuperus et al., 2011 Jung et al., 2007 Park et al., 2002 Schwab et al., 2005 Wu et al., 2009
AP2	*A. thaliana*	At4g36920	Repressor of flowering and floral homeotic gene; a founding member of the AP2-domain transcription factor family	Chen, 2004 Jofuku et al., 1994 Wollmann et al., 2010 Yant et al., 2010
AP2a	*S. lycopersicum*	SGN-U579591	Regulates fruit ripening as part of a negative feedback loop with *CNR*; a likely *AP2* orthologue	Karlova et al., 2011
Gl15	*Z. mays*		Promotes adult phase; AP2-like gene	Lauter et al., 2005 Moose and Sisco, 1996
Ids1/Ts6	*Z. mays*		Specifies determinate spikelet meristem fate; AP2-like gene	Chuck et al., 2007b
Q locus	*T. aestivum*		AP2-like gene; dominant mutations in *Q* contributed to the domestication of wheat	Simons et al., 2006
RAP1	*S. tuberosum*	FM246879	Possible repressor of tuberization and/or flowering; *AP2* homolog	Martin et al., 2009
SMZ	*A. thaliana*	At3g54990	Repressor of flowering	Jung et al., 2007 Mathieu et al., 2009 Schmid et al., 2003 Yant et al., 2010
SNZ	*A. thaliana*	At2g39250	Repressor of flowering	Jung et al., 2007 Mathieu et al., 2009 Schmid et al., 2003 Yant et al., 2010
TOE1	*A. thaliana*	At2g28550	Repressor of flowering	Aukerman and Sakai, 2003 Jung et al., 2007 Mathieu et al., 2009 Wu et al., 2009 Yant et al., 2010
TOE2	*A. thaliana*	At5g60120	Repressor of flowering	Aukerman and Sakai, 2003 Jung et al., 2007 Mathieu et al., 2009 Wu et al., 2009 Yant et al., 2010
TOE3	*A. thaliana*	At5g67180	Repressor of flowering	Yant et al., 2010
Ts4	*Z. mays*		*MIR172e* gene; controls sex determination and meristem cell fate	Chuck et al., 2007b

*Gene identifier under which it can be found in public databases.
Abbreviations: *AP2*, *APETALA2*; *Gl 15*, *Glossy15*; *Ids1*, *Indeterminate spikelet1*; *RAP*, *RELATED TO APETALA2*; *SMZ*, *SCHLAFMÜTZE*; *SNZ*, *SCHNARCHZAPFEN*; *TOE*, *TARGET OF EAT*; *Ts*, *Tasselseed*.

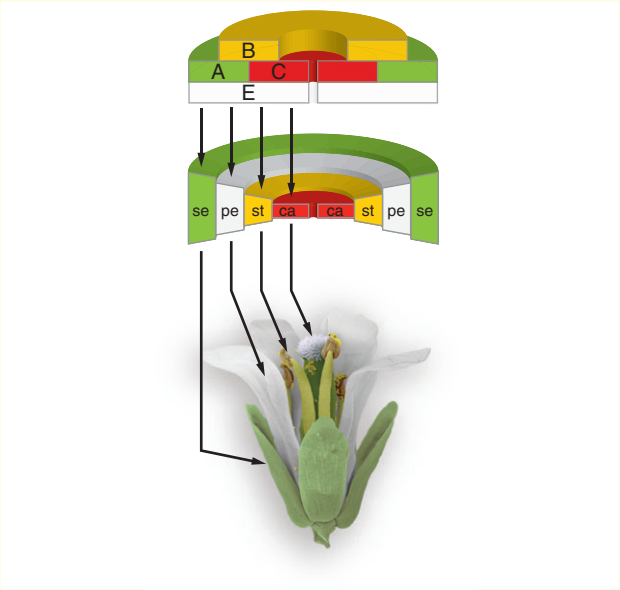
genes, *SEPALLATA1* (*SEP1*), *SEP2*, *SEP3* and *SEP4,* that are required for the pattering of all four whorls of the emerging flower have been identified and have been added to the ABC model as E-class function genes.

Early reports had suggested that *AP2* transcripts could be detected in all parts of the emerging flower (Jofuku et al., 1994), which was puzzling given the A-class function of AP2. However, it has recently been reported that *AP2* expression is actually restricted to the outer two whorls (Wollmann et al., 2010). Interestingly, the expression of miR172 was shown to be restricted to the centre of young floral primordia. As it was recently shown that AP2 can directly bind to and repress the expression of *MIR172b* (Yant et al., 2010), it would appear that AP2 and miR172 are engaged in a negative-feedback loop that presumably helps to establish a sharp boundary between the inner and the outer whorls of the emerging flower (Fig. 3). In this scenario, AP2 not only directly binds to *AG* and prevents its transcription in the outer two whorls, but it is also at the same time cleared from the centre of the floral primordium by the activity of miR172.

miR156/miR172 functions and targets are evolutionary conserved

Both miR156 and miR172 belong to a subset of evolutionary conserved miRNAs that are present throughout the angiosperms (Axtell and Bowman, 2008; Cuperus et al., 2011). However, one of the best-studied miR172 targets is *AP2*, and its orthologues in other species seem to have very divergent roles. This raises the issue of whether the roles of miR156 and miR172 in regulating

Box 2. The ABCE model for flower patterning



The flower of a dicotyledonous angiosperm typically consists of four different organs, the sepals (se), the petals (pe), the stamens (st) and the carpels (ca), that are arranged in concentric whorls (see figure). Analyses of homeotic mutants in which one or more of these floral organs were either lacking or had been replaced by another organ (homeotic transformations) have led to the formulation of the 'classic' ABC model of flower patterning. This model proposes that the combinatorial activity of three genetic functions, A, B and C, are sufficient to specify the four floral organs. According to the ABC model, 'A' on its own specifies sepal fate in the outermost whorl, 'A' and 'B' together determine petals in the second whorl. The stamens in the third whorl are specified by the combinatorial activities of 'B' and 'C', whereas 'C' alone is responsible for the formation of carpels in the centre of the flower. In *Arabidopsis*, A-class activity is conferred by *APETALA1* (*AP1*) and *AP2*, the latter of which is a target of miR172. *AP3* and *PISTILLATA* (*PI*) have B-class function, and C-class function in the centre of the emerging flower is conferred by *AGAMOUS* (*AG*). More recently, the 'ABC' model has been expanded to include a fourth, 'E', function. The E-class genes *SEPALLATA1* (*SEP1*), *SEP2*, *SEP3* and *SEP4* play a crucial role as co-regulators in all four whorls, and underpin the leafy nature of all floral organs.

phase transitions can be generalized to other plant species. Results obtained in maize, rice, tomato, potato and, more recently, in trees, however, indicate that these miRNAs are not only conserved in sequence but also in function.

In maize, mutations that affect phase transitions have been mapped to miR156 and *AP2-like* genes. For example, the dominant maize *Corngrass1* (*Cg1*) mutant affects multiple developmental traits, including leaf initiation and floral architecture. In particular, the *Cg1* mutant initiates more juvenile leaves than do control plants (Chuck et al., 2007a). This was found to be caused by a *STONER* retrotransposon insertion in the upstream regulatory region of a tandem cluster of two miR156 genes, *zma-miR156b* and *zma-miR156c* (Chuck et al., 2007a). Maize contains at least 17 SBP-box genes that are potential targets of miR156 (Hultquist and Dorweiler, 2008); seven of these show reduced expression in *Cg1*. Among them is *teosinte glume architecture 1*, a gene that has been implicated in the domestication of maize (Wang et al., 2005). Another SBP-box gene that is misregulated in *Cg1* is *tasselsheath4*, which is known to regulate the development of bracts and meristem boundaries (Bensen et al., 1995; Chuck et al., 2010). From this, it would appear that the very strong phenotype of the *Cg1* mutant is the result of the reduced expression of several SBP-box genes that regulate various aspects of maize development. Similar to what has been described in miR156-overexpressing *Arabidopsis*, miR172 expression is reduced in the *Cg1* mutant (Chuck et al., 2007a), suggesting that at least some *MIR172* genes are among the targets of the maize SBP-domain proteins. Taken together, these findings have lead to the speculation that changes in the expression levels and temporal/spatial expression patterns of *MIR156* genes and their targets contributed to the evolution of grasses (Chuck et al., 2007a; Wang et al., 2005).

Another gene that regulates the juvenile-to-adult transition in maize is *Glossy15* (*Gl15*). *Gl15* has been cloned and encodes an AP2-like transcription factor that was shown to promote juvenile leaf epidermal traits (Moose and Sisco, 1996). More recently, it has been found that *Gl15* is a target of miR172, and that downregulation of *Gl15* promotes vegetative phase change in maize (Lauter et al., 2005), similar to the way in which downregulation of TOE genes in *Arabidopsis* promotes vegetative phase change (Wu et al., 2009). Besides *Gl15*, another AP2-like gene and target of miR172, *Indeterminate spikelet1* (*Ids1*), which specifies determinate spikelet meristem fate, has been identified in maize. Mutations in the miR172-binding site have been shown to result in the dominant allele of *Ids1*, *tasselseed6* (Chuck et al., 2007b). Furthermore, it has been shown that *Ids1* is the key target of *Tasselseed4*, which represents the *MIR172e* gene of maize (Chuck et al., 2007b). Interestingly, maize *Ids1* is orthologous to the Q locus of wheat, dominant alleles of which have been shown to play an important role in wheat domestication (Simons et al., 2006). Similarly, the maize flowering time quantitative trait locus *Vegetative to generative transition 1* has been mapped to a cis-regulatory region upstream of an AP2-like gene (Salvi et al., 2007). These results from wheat and maize suggest that changes in miRNAs and their targets may have contributed to plant genetic adaptation throughout breeding and evolution.

Interestingly, *MIR172* overexpression has been shown to promote flowering and also tuberization in potato (Martin et al., 2009). Moreover, the effect of miR172 on tuberization has been found to be graft transmissible (Martin et al., 2009). Whether long-distance movement of miR172 itself was responsible for this phenomenon is unclear. The finding that overexpression of *MIR172* in potato roots was insufficient to induce tuberization seems to argue against a non cell-autonomous function of miR172. However, as *MIR172* is expressed in the vasculature of potato and as miR172 has been detected in the phloem exudate from *Brassica napus* (Buhtz et al., 2008), a non-cell-autonomous effect of miR172 on the control of tuberization is still possible.

As mentioned above, morphological differences between the juvenile and the adult phase tend to be more pronounced in woody perennial plants than in annuals such as *Arabidopsis* or maize. Given their slow development, data on the molecular nature of the factors that regulate phase transitions in trees are sparse. However, most recently Wang et al. (Wang et al., 2011) investigated the role of miR156, miR172 and their targets in the control of vegetative phase change in trees. In several species, they observed sequential changes in miR156 and miR172 expression levels, similar to those observed in *Arabidopsis* and maize (Wang et al., 2011). Furthermore, the overexpression of miR156 in a transgenic poplar lead to the reduced expression of SPL genes and of miR172, and drastically prolonged the juvenile phase. From these data it can be concluded that, at least within the angiosperms, miR156/miR172

and their respective targets can be considered to be general regulators of phase transitions, irrespective of whether a plant follows an annual or perennial lifestyle.

miR156 appeared early in land plant evolution, and the mature miRNA has been detected in various mosses, ferns and gymnosperms (Arazi et al., 2005; Axtell and Bartel, 2005; Cuperus et al., 2011; Zhang et al., 2006). The role of miR156 in these plants remains to be determined. In contrast to miR156, miR172 appears to be angiosperm specific and it has not been cloned from other land plants (Axtell and Bowman, 2008; Cuperus et al., 2011), even though the expression of miR172 has been detected by microarrays of RNA extracted from ferns (Axtell and Bartel, 2005) and has been computationally predicted in *Physcomitrella* (Fattash et al., 2007). Regardless of the exact time of miR172 emergence, the question remains as to how miR172 and AP2-like transcription factors were adopted to function in the regulation of phase change. As miR172 apparently arose with the appearance of flowering plants and as miR172/AP2 contribute to patterning the young flower primordium, it seems possible that this reflects the ancient function of this regulatory module.

Conclusions

Over the past few years, two evolutionary conserved miRNAs, miR156 and miR172, and their targets have emerged as key regulators of various phase transitions in plants. Although we have learned a lot about the function of these miRNAs and the genes regulated by their targets, the SPL and AP2-like transcription factors, we know very little about the factors that regulate the temporal and spatial expression of the miRNA genes themselves. For example, levels of miR156 diminish with increasing age of the plant. However, it is largely unclear how plants determine 'age' and how this information is used to regulate gene expression. Identifying the signals and signalling pathways that regulate miR156 expression would not only further our understanding of how plants regulate vegetative phase transitions but would also probably provide important insight into the molecular basics of plant 'aging'.

One characteristic feature of plants is that different shoots on the same plant can exist in different developmental phases, a condition called heteroblasty. In addition, many plants display a remarkable phenotypic plasticity, i.e. the ability to adapt their growth rate and development, allowing them to thrive and survive under varying environmental conditions. However, during any given developmental phase, phenotypic plasticity is limited by homeostatic mechanisms that ensure a minimal degree of developmental robustness. Unfortunately, heteroblasty and plasticity can result in very similar phenotypic outcomes, which has sometimes led to confusion between these two phenomena. However, it is reasonable to assume that the homeostatic mechanisms that limit a plant's plasticity need to be reprogrammed or to be overcome in order to allow developmental phase changes to occur. The recent insight into the molecular mechanisms that shape heteroblastic traits, i.e. the role of miR156, miR172 and their respective targets, will allow us to better discriminate between these two phenomena. At the same time, studying the molecular genetic mechanisms that underlie these phase transitions will lead to a better understanding of what drives plant diversification and distribution in relation to ecological factors.

From a molecular genetic point of view, the most dramatic phase change in the life cycle of plants is probably the diploid to haploid phase change, i.e. between the sporophytic and gametophytic generations. In fact, the ultimate goal of the process of flowering is the production of the gametophytic generation through meiosis in the floral reproductive organs. It will be interesting to see whether central components of the pathways that lead to flowering, i.e. miR156, miR172 and their targets, also contribute to the diploid to haploid phase transition. miR172 appears with the evolution of the angiosperms and it seems unlikely that its ancestral function is in regulating the sporophytic-to-gametophytic phase transition. By contrast, miR156 can be found in all land plants, including mosses, liverworts, ferns and gymnosperms, making it a good candidate for comparative studies.

The function of miRNAs in regulating phase transitions is, of course, not limited to plants. As a matter of fact, the very first miRNAs isolated from *Caenorhabditis elegans*, *lin-4* and *let-7*, have been shown to play important roles during *C. elegans* post-embryonic development (Kato and Slack, 2008; Vella and Slack, 2005). In brief, *lin-4* and one of the targets of *lin-4*, *LIN-14*, are required for the larval stage 1 (L1)-to-L2 transition. Similarly, *let-7* and its key target *LIN-41* are essential for the L4-to-adult transition. *lin-4* and *let-7* belong to a subgroup of evolutionary highly conserved miRNAs, which is indicative of a conserved function. Interestingly, both *lin-4* and *let-7* need to be upregulated during development to allow phase transitions to occur. This temporal regulation resembles that of miR172 in plants. Although the observation that miRNAs play a role in phase transitions in the different kingdoms is an interesting one, it begs the question as to what it is that makes miRNAs so particularly useful for controlling phase changes and developmental timing? One hypothesis is that the temporal activation of miRNAs results in a rapid clearing of their target mRNA from cells, allowing for a swift and irreversible phase transition. Another possibility is that organisms employ miRNAs against regulators of phase transition to confer robustness to the developmental programs between the transitions. This may be achieved by repressing leaky transcripts in the case of high miRNA to target ratios or to buffer against fluctuations in target expression in the case of co-expression at intermediate levels (Hornstein and Shomron, 2006). The latter scenario, in particular, may enable organisms to manage duplication events or altered expression patterns that might occur in the course of the evolution of key developmental control genes, the altered expression levels of which would otherwise be detrimental. The co-evolution of such key developmental genes with their targeting miRNAs would allow more gradual changes and could open the opportunity for the evolution of new varieties and speciation to evolve. Clearly, no matter the particular biological system we are working on, we can still expect many exiting new and surprising findings from the analysis of miRNAs and their role in regulating phase transitions.

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Competing interests statement

The authors declare no competing financial interests.

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