

Coming into bloom: the specification of floral meristems

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In flowering plants, the founder cells from which reproductive organs form reside in structures called floral meristems. Recent molecular genetic studies have revealed that the specification of floral meristems is tightly controlled by regulatory networks that underpin several coordinated programmes, from the integration of flowering signals to floral organ formation. A notable feature of certain regulatory genes that have been newly implicated in the acquisition and maintenance of floral meristem identity is their conservation across diverse groups of flowering plants. This review provides an overview of the molecular mechanisms that underlie floral meristem specification in *Arabidopsis thaliana* and, where appropriate, discusses the conservation and divergence of these mechanisms across plant species.

Introduction

Flowering plants, also known as angiosperms (see Glossary, Box 1), were the last of the seed-bearing plant groups to evolve. The most obvious features that distinguish angiosperms from other seed-bearing plants are their reproductive organs, the flowers. In the course of flowering, plants undergo a transition from vegetative to reproductive growth (see Glossary, Box 1), known as the floral transition. Flowers contain reproductive structures, such as stamens and carpels (see Glossary, Box 1; see also Fig. 1), and upon fertilization a subset of carpels develops into fruits. These fruits contain seeds, from which new plants can grow, thus permitting the transfer of genetic information to the next generation.

When plants initiate flowering, the vegetative shoot apical meristem (SAM; see Glossary, Box 1), which gives rise to all the parts of a plant that are above ground, is transformed into an inflorescence meristem (IM; see Glossary, Box 1). The IM, in turn, generates a collection of undifferentiated cells called floral meristems (FMs) that give rise to floral organs. As FMs arise in response to multiple flowering signals and eventually differentiate into various types of floral organ, the regulation of FM development is a crucial and dynamic switch that allows for the successful reproductive development of flowering plants in an unpredictable environment.

Morphological changes of flower development have been monitored in detail in the model plant *Arabidopsis thaliana* (Smyth et al., 1990), in which flower development is divided into 12 stages according to a series of landmark events. Floral primordia that are present prior to visible floral organogenesis are generally considered to be FMs. Even though floral anlagen are morphologically invisible before stage 1, they have already become distinguishable from other cells in IMs, as is apparent from the expression of certain marker genes. One such marker gene is *AINTEGUMENTA* (*ANT*), which encodes a transcription factor of the plant-specific AP2/EREBP family (Fig. 2A). Floral anlagen at this transitional phase are usually

Box 1. Glossary

Abaxial Facing away from the axis of the stem; also the lower surface of leaves.

Adaxial Facing towards or adjacent to the axis of the stem; also the upper surface of leaves.

Angiosperm A flowering plant in which ovules (seeds) are enclosed in an ovary (fruit).

Axillary meristem The meristematic tissue located in the upper angle between a leaf and a stem.

Carpel Female reproductive organ, consisting of a pollen-receiving part, the stigma, a stalk-like structure, the style, and the ovule-containing ovary.

Cotyledon The first leaf or leaves generated from a seed-bearing plant embryo.

Dicotyledon A flowering plant with two cotyledons and flower parts in multiples of four or five.

Eudicotyledon Regarded as a 'true' dicotyledon that typically shares the same characteristics as a dicotyledon, but that has three or more pores in its pollen.

Gibberellin A plant hormone that influences various developmental processes, including growth stimulation, germination and flowering.

Indeterminacy The ability to continue to grow indefinitely.

Inflorescence A shoot that contains a cluster of flowers.

Meristem A plant tissue that consists of undifferentiated cells with growth potential.

Monocotyledon A flowering plant with a single seed leaf (cotyledon) and flower parts in multiples of three.

Pedicel The stalk of an individual flower.

Petal A modified leaf that forms part of a flower and is usually brightly coloured.

Photoperiod Length of light and darkness in one day.

Reproductive growth The mature phase of a flowering plant; the plant has reproductive organs (flowers).

Sepal Outermost leaf-like structure of a flower that often serves as protection.

Shoot apical meristem The meristematic tissue at the tip of a plant shoot.

Spikelet Basic leaf-like unit of the inflorescence of grasses, enclosing one or more florets.

Stamen Male reproductive organ, consisting of a stalk, termed the filament, and a pollen-containing structure, the anther.

Vegetative growth The non-reproductive, growing phase of the life cycle of a flowering plant; after the seedling phase but before the floral transition.

Vernalization Prolonged exposure to cold temperatures that some plants require to become competent to flower.

Whorl Arrangement of structures in a circle around an axis.

referred to as stage 0 FMs (Long and Barton, 2000). Stage 1 FMs emerge as outward bulges on the flank of the IM, with each new FM forming at an angle of ~130°-150° to previously established ones. From stage 1 to the end of stage 2, FMs enlarge gradually into ball-shaped structures and become separated from the IM (Fig. 2B). The primordia of the first whorl (see Glossary, Box 1) of floral organs, sepals (see Glossary, Box 1), appear at the periphery of the FMs at stage 3 and start to overlie FMs at stage 4, and this is followed by the successive emergence of other floral organs in the internal whorls.

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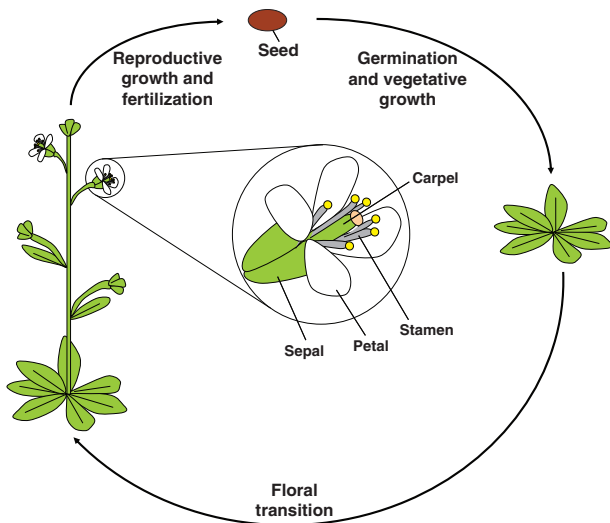


Fig. 1. *Arabidopsis thaliana* life cycle and flower architecture.

After seed germination, the young seedling grows in size during the vegetative phase. Upon receiving appropriate environmental and endogenous signals, the plant undergoes the floral transition, the change from vegetative to reproductive growth, which results in the continuous formation of flowers in the bolting inflorescence. A typical flower consists of sepals, petals, stamens and carpels (from the outermost to the innermost whorls). Usually, sepals protect the fragile flower, whereas petals attract insects for pollination through their bright colours. The male organs, stamens, and the female organs, carpels, are responsible for fertilization and for the generation of offspring through the production of viable seeds.

The intimate developmental link between FMs and floral organs indicates that the specification of FMs is a key preliminary step for successful flower development.

Recent molecular genetic studies have provided new insights into the specification of FMs in *Arabidopsis* and other flowering plants. In this review, we focus on the latest progress in our understanding of the regulatory networks that underpin several coordinated programmes of FM development in *Arabidopsis* and discuss the homologues of key genes that regulate FMs in a variety of flowering plants (angiosperms) to evaluate the conservation of relevant mechanisms across plant species.

Setting the scene: IM formation

FMs are exclusively produced from IMs, the reproductive SAMs into which vegetative SAMs are transformed during the floral transition. By contrast, other organ primordia initiate from plant SAMs during both vegetative and reproductive phases; this suggests a unique role for IMs in specifying FMs. It should, however, be noted that many grasses have evolved more specialized, so-called axillary meristems (see Glossary, Box 1) from IMs that are produced before producing FMs to acquire highly branched inflorescences (Fig. 2C). For instance, indeterminate IMs (see Glossary, Box 1) or their derived branch meristems in maize (*Zea mays*) give rise to spikelet pair meristems (see Glossary, Box 1), which further differentiate into spikelet meristems and finally into FMs (Barazesh and McSteen, 2008). Regardless of how FMs are ultimately formed, however, the generation of IMs is a prerequisite for FM specification in most flowering plants.

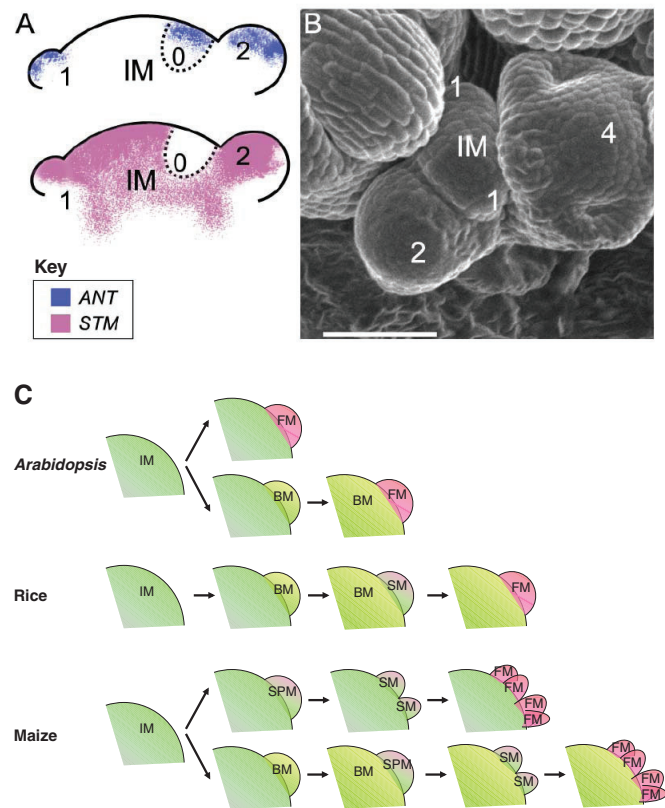


Fig. 2. *Arabidopsis* floral meristem development. (A) Schematic of marker gene expression in early floral meristems (FMs). The stages of emerging FMs are indicated as 0, 1 or 2 (Smyth et al., 1990). In the stage 0 FM, which is also called the floral anlage, the transcription factor *AINTEGUMENTA* (*ANT*), which belongs to the plant-specific AP2/EREBP family of transcription factors, is expressed in the peripheral region, whereas the homeobox gene *SHOOT MERISTEMLESS* (*STM*) is not expressed (Long and Barton, 2000). (B) Scanning electron micrograph of the top view of an *Arabidopsis* inflorescence meristem (IM). The stages of emerging FMs are indicated as 1, 2 or 4 (Smyth et al., 1990). Scale bar: 100 μ m. (C) Developmental routes from IMs to FMs in *Arabidopsis*, rice and maize. In *Arabidopsis*, FMs are produced either directly from primary IMs or from branch meristems (BMs; also called secondary IMs) that are derived from primary IMs. In rice, the main inflorescences terminate after the generation of several lateral branches. Each BM gives rise to spikelet meristems (SMs), each of which eventually produces a single FM. In maize, primary IMs produce spikelet pair meristems (SPMs) or BMs that further differentiate into SPMs. Each SPM produces two SMs, each of which gives rise to two FMs.

The molecular mechanisms that underlie the transition from vegetative SAMs to IMs have been intensively investigated in *Arabidopsis* (Fig. 3). This transition is mediated by a complex network of genetic pathways that regulate flowering in response to environmental and developmental signals (Blazquez et al., 2003; Boss et al., 2004; Mouradov et al., 2002; Simpson and Dean, 2002). The autonomous pathway regulates flowering by monitoring endogenous cues from different developmental stages, whereas the gibberellin (GA; see Glossary, Box 1) pathway affects flowering particularly in short-day conditions. The photoperiod and vernalization pathways (see Glossary, Box 1) mediate the responses to environmental signals, such as day length and low temperatures. In addition, some other genetic pathways, such as the ones that

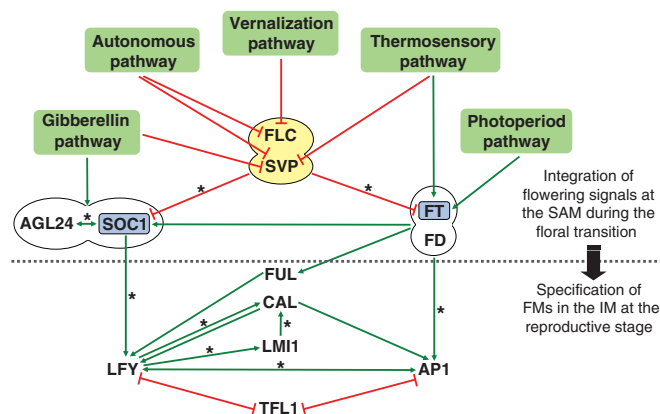


Fig. 3. Regulation of FM identity. FM identity is regulated through the integration of multiple flowering signals, with the floral pathway integrators *SOC1* and *FT* (blue) perceiving environmental and developmental signals through several flowering genetic pathways. During the floral transition, the *FLC-SVP* complex (yellow) represses *SOC1* expression in the leaf and SAM and *FT* expression in the leaf, whereas the *FT-FD* complex promotes the expression of *SOC1*, *AP1*, and probably *FUL* in the SAM. *SOC1* and *AGL24* directly upregulate the expression of one another and also form a protein complex, which is localized at the SAM. In the IM, the increased activity of *SOC1* and *FT* promotes the expression of several FM identity genes, including *LFY*, *AP1*, *CAL* and *FUL*, which in turn specify FM identity on the flanks of the IM. Green arrows indicate promoting effects, whereas red linkers indicate repressive effects. Two linked ellipses indicate protein-protein interactions. Asterisks indicate direct transcriptional regulation. *AGL24*, *AGAMOUS-LIKE 24*; *AP1*, *APETALA1*; *CAL*, *CAULIFLOWER*; *FLC*, *FLOWERING LOCUS C*; *FM*, floral meristem; *FT*, *FLOWERING LOCUS T*; *FUL*, *FRUITFULL*; *IM*, inflorescence meristem; *LFY*, *LEAFY*; *SAM*, shoot apical meristem; *SOC1*, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*; *SVP*, *SHORT VEGETATIVE PHASE*.

respond to changes in light quality and ambient temperature, have been proposed to affect flowering. The flowering signals perceived by these pathways converge on the transcriptional regulation of two major floral pathway integrators, *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*). These, in turn, activate FM identity genes, such as *LEAFY* (*LFY*) and *APETALA1* (*AP1*), to produce FMs on the flanks of IMs (Blazquez and Weigel, 2000; Kardailsky et al., 1999; Kobayashi et al., 1999; Lee et al., 2000; Lee et al., 2008; Liu et al., 2008; Samach et al., 2000).

The integration of flowering signals is tightly controlled by a repressor complex that consists of two MADS-box transcription factors, *FLOWERING LOCUS C* (*FLC*) and *SHORT VEGETATIVE PHASE* (*SVP*) (Hartmann et al., 2000; Li et al., 2008; Michaels and Amasino, 1999; Sheldon et al., 1999). The vernalization and autonomous pathways mainly repress *FLC* expression through the modulation of its chromatin structure (Michaels, 2009), which promotes flowering by antagonizing the repressive effect of *FLC* on *FT* and *SOC1* expression (Helliwell et al., 2006; Searle et al., 2006). *FLC* represses *FT* expression in leaves; this blocks the translocation of the systemic flowering signals that contain *FT* protein to the SAMs, an event that is required for activating the expression of *SOC1* and *AP1* (Abe et al., 2005; Corbesier et al., 2007; Searle et al., 2006; Wigge et al., 2005). *FLC* also directly represses the expression of *SOC1* and of the *FT* cofactor *FD* in SAMs (Searle et al., 2006), thus further inhibiting the meristem response to flowering signals.

In vegetative seedlings at various ages, the *FLC-SVP* repressor complex responds mainly to flowering signals that are perceived by the autonomous, the thermosensory and the GA pathways (Hartmann et al., 2000; Lee et al., 2007b; Li et al., 2008). Their mutually dependent function directly regulates *SOC1* expression in whole seedlings, as well as *FT* expression in leaves. Thus, most flowering pathways (with the exception of the photoperiod pathway) appear to promote the expression of *FT* and *SOC1* predominantly through derepression mechanisms (Fig. 3).

Unlike *FT*, *SOC1* is highly expressed in IMs, which makes it a good candidate for contributing to the spatial specificity of FM initiation, during which a small block of cells (normally four cells) on the IM flank acquire progenitor fate for a future FM (Bossinger and Smyth, 1996; Lee et al., 2000; Samach et al., 2000). By contrast, *SVP* is expressed in both leaves and SAMs during the vegetative phase and is absent from IMs during the reproductive phase (Hartmann et al., 2000). As the repressive effect of *SVP* on *SOC1* transcription outweighs the effects of *SOC1* activators such as *FT* and *AGAMOUS-LIKE 24* (*AGL24*) (Li et al., 2008), a decrease in *SVP* expression is a key event required for the transformation of vegetative SAMs into IMs. The abundance of *SVP* protein has been found to increase in certain circadian clock mutants under continuous light (Fujiwara et al., 2008), but how *SVP* expression is gradually downregulated in SAMs during floral transition remains unclear. Overall, the interaction of the above-mentioned flowering regulators in various flowering genetic pathways mediates the transition from vegetative SAMs to IMs, from which FMs are derived.

Protruding out: FM initiation

The regulation of FM initiation not only involves the activation of two well-known FM identity genes, *LFY* and *AP1*, but also depends on the control of auxin flux and tissue polarity (Blazquez et al., 2006). Even though the latter two factors have seldom been reviewed in association with FM specification, they are temporally and spatially correlated to the onset of FM development (Blazquez et al., 2006; Heisler et al., 2005). In this section, we discuss how the distribution of auxin, which is affected by its biosynthesis, transport and signalling, influences FM initiation in *Arabidopsis* and monocotyledons (see Glossary, Box 1; see also Fig. 4). In addition, we also review the regulation of tissue polarity during FM initiation.

Mechanisms of FM initiation

In *Arabidopsis*, the heterogeneous distribution of auxin affects the initiation of all axillary meristems (Benkova et al., 2003), including the initiation of FMs in IMs. Here, auxin accumulates at the positions of floral anlagen, but gradually decreases in concentration with increasing distance from them (Heisler et al., 2005; Oka et al., 1999; Reinhardt et al., 2003). This pattern of auxin distribution is mediated by both auxin biosynthesis and polar auxin transport. At the early stages of reproductive development, FM formation is abolished in *Arabidopsis* quadruple mutants (*yuc1 yuc2 yuc4 yuc6*) of the *YUCCA* (*YUC*) family of flavin monooxygenases, which are essential for auxin biosynthesis (Cheng et al., 2006). Simultaneous mutations in these four *YUC* genes result in a naked inflorescence stem. Similar phenotypes are seen in plants with loss-of-function mutations in *NAKED PINS IN YUC MUTANTS* (*NPY*) and *AGC KINASE* genes. Although these genes have been proposed to act in a linear pathway together with *YUC* genes (Cheng et al., 2008), their exact function in auxin-mediated organogenesis remains to be elucidated further. Loss-of-function mutations in the auxin efflux carrier *PIN-FORMED 1* (*PINI*), which regulates polar auxin transport, also produce naked inflorescence stems without FMs

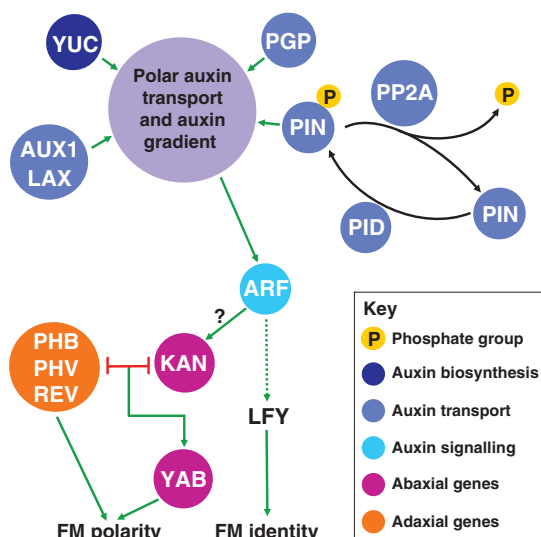


Fig. 4. Regulation of FM initiation by auxin and tissue polarity. Proteins involved in auxin biosynthesis (YUCs), transport (PINs, PGPs, AUX1, LAXs, PID, PP2A) and signalling (ARFs) coordinate the polarized auxin distribution that affects FM initiation in the IM, probably through the regulation of LFY activity and tissue polarity. The interaction between adaxial-fate-promoting regulators (PHB, PHV and REV) and abaxial-fate-promoting regulators (KANs and YABs) establishes a tissue polarity that might contribute to proper FM initiation. The link between auxin signalling and FM polarity, which could be mediated by the interaction between ARFs and abaxial-promoting regulators, has not yet been elucidated (indicated by a question mark). The hypothetical regulation of LFY by ARF is indicated by a dotted line. Green arrows indicate promoting effects, whereas red linkers indicate repressive effects. ARF, AUXIN RESPONSE FACTOR; AUX1, AUXIN RESISTANT 1; FM, floral meristem; KAN, KANADI; LAX, LIKE AUX1; PGP, P-glycoprotein; PID, PINOID; PIN, PIN-FORMED; PP2A, PROTEIN PHOSPHATASE 2A; REV, REVOLUTA; PHB, PHABULOSA; PHV, PHAVOLUTA; YAB, YABBY; YUC, YUCCA.

(Vernoux et al., 2000). The live imaging of *Arabidopsis* IMs with concurrent monitoring of the expression of PIN1 and of the auxin-responsive reporter DR5 has further revealed that auxin transport is intimately associated with FM initiation (Heisler et al., 2005).

The regulation of PIN1 activity also affects FM initiation. Intercellular auxin fluxes are controlled by the phosphorylation status of PINs, which is mediated through the antagonistic regulation of an AGC kinase, PINOID (PID), and PROTEIN PHOSPHATASE 2A (PP2A) (Michniewicz et al., 2007). As *pid* mutants fail to produce FMs (Cheng et al., 2008), the modulation of the PIN1 phosphorylation status appears to play a role in FM initiation. Interestingly, additional factors that regulate PIN1 function have been identified recently. For example, P-glycoprotein (PGP) transport proteins, which have been suggested to form another group of auxin efflux carriers, genetically interact with PINs in a concerted fashion during organogenesis (Mravec et al., 2008), whereas AUXIN RESISTANT 1 (AUX1), an auxin influx carrier, and its paralogues LIKE AUX 1, 2 and 3 (LAX1, LAX2 and LAX3) are required for mediating coordinated PIN1 polarization (Bainbridge et al., 2008). Whether these factors are also involved in PIN1-mediated FM initiation, however, remains to be elucidated.

Consistent with the roles of auxin biosynthesis and transport in FM initiation discussed above, auxin signalling also has a crucial function in this process. Auxin response factors (ARFs) are

considered to be key components of the auxin signalling pathway, and loss-of-function mutations in the ARF gene *MONOPTEROS* (also known as *ARF5*) abolish FM initiation (Przemeck et al., 1996). The phenotype seen in these mutants is similar to that observed in *yuc*, *pin1* and *pid* mutant plants. These results clearly show that auxin plays an indispensable role in FM initiation.

Several lines of evidence have provided a molecular link between auxin and FM specification. First, *LFY* expression is reduced and changed into a ring-like pattern that encircles the IM of *pin1* mutants, and the expression of *LFY* downstream targets, such as *API* and *AP3*, also decreases in *pin1* (Vernoux et al., 2000). Second, the dynamic expression of PIN1 protein corresponds to *LFY* expression at the sites of FM initiation (Heisler et al., 2005). Third, an auxin response element has been identified in the *LFY* promoter that might be recognized by an ARF (Bai and DeMason, 2008). Taken together, these observations indicate that the initiation of FMs, which is regulated by auxin, might be integrated with the specification of FM identity by *LFY*.

Conservation of FM initiation mechanisms

Recent progress suggests that the regulatory mechanisms of FM initiation through auxin biosynthesis and transport might be partially conserved from *Arabidopsis* to monocotyledons (see Glossary, Box 1). The maize gene *sparse inflorescence 1* (*spi1*) encodes a YUC-like flavin monooxygenase that is involved in local auxin biosynthesis and in the regulation of axillary meristems, including the initiation of spikelet meristems and FMs (Gallavotti et al., 2008a). In addition, *PIN1*-like genes have been identified in maize and rice (Carraro et al., 2006; Paponov et al., 2005). *Zea mays* PIN1a (ZmPIN1a), a PIN1 homologue in maize, is localized in the L1 layer of axillary meristems and IMs (Gallavotti et al., 2008b), which is comparable to the localization of PIN1 in *Arabidopsis*. Moreover, ZmPIN1a activity rescues *Arabidopsis pin1-3*, resulting in the re-establishment of auxin maxima and the re-formation of FMs, which indicates that the auxin transport mechanism during FM initiation might be conserved between *Arabidopsis* and grasses (Gallavotti et al., 2008b). Interestingly, the phosphorylation and localization of ZmPIN1a is also regulated by a homologue of PID, BARREN INFLORESCENCE2 (BIF2) (McSteen et al., 2007; Skirpan et al., 2009), which suggests similarities in the regulation of auxin transporter trafficking between maize and *Arabidopsis*. In rice (*Oryza sativa*), OsPID, another orthologue of PID, has been suggested to function in polar auxin transport (Morita and Kyozyuka, 2007), but its role in FM initiation is so far unknown.

Tissue polarization during FM initiation

FM initiation inherently involves the establishment of tissue polarity, as illustrated by the fact that several polarity genes were found to mark the abaxial and adaxial sides (see Glossary, Box 1) of FMs. The *Arabidopsis* *FILAMENTOUS FLOWER* (*FIL*) gene, which encodes a member of the YABBY family of transcription factors, is specifically expressed on the abaxial side of emerging FMs (Sawa et al., 1999b; Siegfried et al., 1999). In *fil* mutants, FMs differentiate into various structures, including flowerless pedicels (see Glossary, Box 1) and curled sepals (Chen et al., 1999). Moreover, crossing *fil* mutants with *ap1* or *lfy* mutants results in plants with enhanced defects in FM formation (Sawa et al., 1999a). These observations suggest that properly established tissue polarity regulated by *FIL* is required, together with *API* and *LFY*, for FM specification.

It has been shown that the initial asymmetric development of leaf primordia is controlled by a mutual antagonism between the *PHABULOSA* (*PHB*)-like genes, which promote adaxial cell fate,

and the abaxial-fate-promoting *KANADI* (*KAN*) genes. This antagonism, in turn, affects polar *YABBY* expression, which promotes abaxial cell fate (Eshed et al., 2001; Eshed et al., 2004). Whether this mechanism also regulates the function of *FIL* in FMs remains unknown. *PHB*, *PHAVOLUTA* (*PHV*) and *REVOLUTA* (*REV*) are a group of class III homeodomain/leucine zipper (HD-ZIP) genes that regulate adaxial cell fate in lateral organs (Emery et al., 2003; McConnell et al., 2001). Among these genes, *REV* has been demonstrated to play an important role in FM formation (Otsuga et al., 2001). In *rev* mutants, some FMs develop with reduced size. Notably, *fil rev* double mutants show greatly enhanced floral defects, with FMs transforming completely into pedicels (Chen et al., 1999). Thus, the interaction between adaxial-promoting genes, such as *REV*, and abaxial-promoting genes, like *FIL*, might determine tissue polarity in a way that is important for the proper initiation of FMs.

Interestingly, *ETTIN*, which is also known as *AUXIN RESPONSE TRANSCRIPTION FACTOR 3* (*ARF3*), regulates organ asymmetry through the modulation of *KAN* activity (Pekker et al., 2005). This links auxin signalling with the regulation of tissue polarity and indicates that tissue polarity is fine-tuned through certain ARFs that are stimulated by auxin gradients. Furthermore, during FM initiation, *PIN1* expression marks a domain between abaxial and adaxial cell identities, as marked by *FIL* and *REV* expression, respectively. This lends further support to the notion that auxin transport patterns influence organ polarity in FMs (Heisler et al., 2005). It will be instructive to investigate how auxin is involved in FM initiation. One possibility is that it affects FM identity through *LFY* and mediates FM polarity by regulating the expression of abaxial and adaxial genes (Fig. 4).

Acquisition of FM identity

The emerging FMs are specified by the so-called FM identity genes, including *LFY* and *API*. The characterization of FM identity genes in *Arabidopsis* and the isolation of their homologues in different plant species suggest that some conserved mechanisms underlie FM specification, even though the homologues of FM identity genes might have evolved various functions in different taxonomic groups.

Regulation of *LFY* and *API*

LFY and *API* are two major FM regulators that specify FM identity on the flanks of IMs in *Arabidopsis* (Bowman et al., 1993; Mandel and Yanofsky, 1995; Weigel et al., 1992). When the activity of either gene is lost, FMs that would normally develop into flowers are partly converted into IMs. It has long been known that the shoot identity gene *TERMINAL FLOWER 1* (*TFL1*) antagonizes *LFY* and *API* and thus counteracts the establishment of FM identity (Liljegren et al., 1999; Ratcliffe et al., 1999). However, this antagonistic interaction does not explain the puzzle of how *LFY* and *API* are regulated in response to upstream flowering signals to specify FMs in IMs, as the mechanism by which *TFL1* is integrated into the flowering regulatory networks remains unclear. Recent studies on the integration of flowering signals have, however, shed some light on the regulation of *LFY* and *API* (Fig. 3).

LFY plays a dual role in regulating FM identity and floral organ patterning (Parcy et al., 1998), and its expression is affected by several flowering pathways (Blazquez and Weigel, 2000). Among all the known flowering-time factors, *SOC1* is currently the only transcription factor known to bind to the *LFY* promoter in vivo, and this binding process is partly mediated through the interaction of *SOC1* with *AGL24* (Lee et al., 2008; Liu et al., 2008). *SOC1* expression gradually increases in SAMs during the floral transition

in response to multiple flowering signals (Lee et al., 2000; Samach et al., 2000). This increase could provide temporal and spatial cues for promoting *LFY* expression in the incipient floral primordia to the threshold levels that are required for FM specification.

Three closely related MADS-box genes, *API*, *CAULIFLOWER* (*CAL*) and *FRUITFULL* (*FUL*), also appear to be potential activators of *LFY* during the floral transition (Ferrandiz et al., 2000). A combination of mutations in these three genes produces leafy shoots in place of flowers (Ferrandiz et al., 2000). The abolishment of *LFY* upregulation is partially responsible for this phenotype, which indicates that *FUL*, *API* and *CAL* act redundantly upstream of *LFY* in determining FM identity. The functional redundancy between *FUL* and *SOC1* also masks their roles in FM formation (Melzer et al., 2008). These two genes share a similar expression pattern in both IMs and FMs. *soc1 ful* double mutant plants show strongly delayed flowering when grown under long day conditions when compared with the single mutants. Interestingly, the apical IMs of *soc1 ful* revert into vegetative SAMs after the plants enter the reproductive phase (Melzer et al., 2008). This pattern is recurrent, which is reminiscent of the lifestyle of perennial plants. These observations demonstrate that *SOC1* and *FUL* not only control flowering time, but also play an important role in meristem determinacy, which might be partly attributed to their function in modulating *LFY* expression. Another key floral pathway integrator, FT, and its cofactor, FD, activate *SOC1* expression in IMs (Abe et al., 2005; Corbesier et al., 2007; Wigge et al., 2005) and promote *FUL* expression in leaves as well as, potentially, in IMs (Teper-Bamnolker and Samach, 2005). Therefore, FT could control *LFY* expression through both *SOC1* and *FUL* during the floral transition.

API itself is another major FM identity gene that is specifically expressed in emerging FMs (Mandel et al., 1992). During the floral transition, *API* expression is directly activated by *LFY* and by a complex consisting of FT and FD (Abe et al., 2005; Wagner et al., 1999; Wigge et al., 2005). *API* function overlaps with that of *CAL* genes, as *apl1 cal1* mutants show a complete transformation of FMs into IMs (Bowman et al., 1993). *LFY* determines FM identity by directly controlling the expression of at least three transcription factors, namely *API*, *CAL*, and *LATE MERISTEM IDENTITY 1* (*LMII*), which encodes a class I HD-ZIP transcription factor (Saddic et al., 2006; William et al., 2004). Together with *LFY*, *LMII* controls *CAL* expression directly. This interaction is suggested to form a coherent feed-forward loop that fine-tunes the FM identity switch in response to environmental stimuli (Saddic et al., 2006). These data suggest that the network that converges on the regulation of *LFY* and *API* by *SOC1* and *FT* might be an essential molecular link that translates the multiple flowering signals integrated by *FT* and *SOC1* into the actual specification of FMs by *LFY* and *API* (Fig. 3).

Homologues of *LFY* and *API*

Since the isolation of the *LFY* homologue *FLORICAULA* (*FLO*) and of the *API* homologue *SQUAMOSA* (*SQUA*) in snapdragon (*Antirrhinum majus*) (Coen et al., 1990; Huijser et al., 1992), additional homologues of *LFY* and *API* have been identified in many other plant species. *LFY* homologues are present in all the land plants that have been analyzed to date, including moss (Chujo et al., 2003). A *LFY* homologue appears to have been recruited to flower development in the ancestor of all angiosperms, as it is involved in this process in all angiosperm species tested so far (Benlloch et al., 2007; Blazquez et al., 2006). The extent of phenotypical complementation of *Arabidopsis lfy* mutants by different *LFY* homologues seems to be related to the taxonomic distance from *Arabidopsis*, ranging from no complementation by moss

homologues to full complementation by angiosperm homologues (Maizel et al., 2005). In some instances, however, *LFY* homologues seem to have been recruited to play additional roles along with their conserved function in FM specification. For example, some *LFY* homologues, such as *UNIFOLIATA* in pea and *FALSIFLORA* in tomato, regulate leaf development (Hofer et al., 1997; Molinero-Rosales et al., 1999), whereas studies on the function of maize and rice *FLO/LFY* genes have revealed a role for monocotyledonous *LFY* homologs in inflorescence branching (Bomblies et al., 2003; Kyoizuka et al., 1998).

Phylogenetic analyses of *API/FUL*-like MADS-box genes reveal the presence of two gene clades within the core eudicotyledons (see Glossary, Box 1), eu*API* (e.g. *API*) and eu*FUL* (e.g. *FUL*) (Litt and Irish, 2003). The homologues of the eu*API* gene clade are found only in core eudicotyledons, which includes the majority of extant angiosperm species (Litt and Irish, 2003). This suggests that eu*API* function might be specific to flower formation in core eudicotyledons. Similar to *LFY* homologues, however, some homologues of *FUL*-like genes, which are not restricted to eudicotyledons, show novel functions in certain plant species in addition to their role in specifying FM identity (Benlloch et al., 2007). In grasses, for example, *FUL1* and *FUL2* have evolved additional functions in regulating the floral transition (Preston and Kellogg, 2007).

Grass meristem identity genes

Apart from the homologues of *Arabidopsis* FM identity genes, other meristem identity genes that are unique to grass species have been isolated. FM initiation in maize is controlled by an *APETALA2* (*AP2*)-like gene, *indeterminate spikelet1* (*ids1*), and by its related gene *sister of indeterminate spikelet1* (*sid1*). Loss-of-function mutations in either of these genes abolish FM initiation, which indicates that in grasses, the *AP2* genes might replace *LFY* to function in FM identity (Chuck et al., 2008). Therefore, the mechanisms that underlie the specification of FMs in grasses are partly similar to those in *Arabidopsis*; however, grasses, which frequently have complex floral and inflorescence structures, might also have evolved some unique genetic and molecular programmes of FM specification.

Maintenance of FM identity

In the course of flowering, the emerging FMs can potentially take a developmental step backwards to turn into inflorescence shoots, a phenomenon called floral reversion, or precociously differentiate to produce abnormal floral organs. Therefore, simply establishing FM identity is not sufficient for securing normal flower development. Additional mechanisms that are responsible for the active maintenance of floral identity in FMs appear to be required until normal floral patterning occurs at a later stage. In this section, we discuss the evidence in favour of the existence of such mechanisms.

Repression of floral reversion

In *Arabidopsis*, floral reversion often occurs in FM identity mutants, such as *lfy* and *ap1*, which indicates that the mutated genes play key roles in maintaining FM identity by repressing floral reversion.

Regulation of *AGL24*, *SVP* and *SOC1*

ap1 mutants are characterized by the generation of secondary flowers or inflorescences in individual FMs, which signifies a partial reversion from FMs to IMs (Bowman et al., 1993). These phenotypes appear to be partially attributable to the activity of three flowering-time genes, *AGL24*, *SVP* and *SOC1* (Liu et al., 2007; Yu

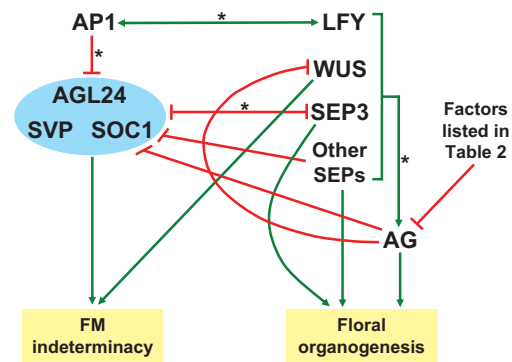


Fig. 5. Maintenance of FM identity. FM identity is maintained through a balance between FM indeterminacy and differentiation. The activation of floral homeotic genes (e.g. *AG*) in FMs requires the activity of *LFY*, *WUS* and *SEPs*. In FMs prior to stage 3, *AG* is not activated because the expression of one of its upstream regulators, *SEP3*, is repressed by the flowering-time genes *SVP*, *SOC1* and *AGL24* (blue), the expression of which is controlled by *AP1* and *SEPs*. By stage 3, the repression of *SVP*, *SOC1* and *AGL24* by *AP1* and *SEPs* derepresses *SEP3*, which, together with *LFY*, *WUS* and other *SEPs*, activates *AG*. *SVP*, *SOC1*, *AGL24* and *WUS* promote FM indeterminacy, whereas floral homeotic genes (e.g. *AG* and *SEPs*) promote floral organogenesis. Several regulatory feedback loops regulate FM homeostasis to mediate the transition from FM indeterminacy to differentiation. Green arrows indicate promoting effects, whereas red linkers indicate repressive effects. Asterisks indicate direct transcriptional regulation. *AG*, *AGAMOUS*; *AGL24*, *AGAMOUS-LIKE 24*; *AP1*, *APETALA1*; FM, floral meristem; *LFY*, *LEAFY*; *SEP*, *SEPALLATA*; *SOC1*, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*; *WUS*, *WUSCHEL*.

et al., 2004), because loss-of-function mutations in these three genes, either individually or combined, alleviate the FM defects seen in *ap1* by lowering the frequency of secondary structure production. Indeed, the expression of these genes is upregulated in *ap1* FMs. Consistently, the transgenic expression of *AGL24* under the control of the constitutive 35S promoter (*35S:AGL24*) promotes the transformation of FMs into IMs, a phenotype that is enhanced by *35S:SOC1*, whereas the transgenic expression of *35S:SVP* promotes the transformation of FMs into vegetative shoots (Liu et al., 2007; Masiero et al., 2004; Yu et al., 2004). It has been shown that induced *AP1* activity represses the expression of *AGL24*, *SVP* and *SOC1* (Liu et al., 2007; Wellmer et al., 2006; Yu et al., 2004), and that *AP1* binds directly to the promoters of these three genes (Gregis et al., 2008; Liu et al., 2007). These results suggest that the suppression of these flowering-time genes by *AP1* is one of the processes involved in maintaining FM identity (Fig. 5).

In contrast to *AP1*, *LFY* might not directly repress *AGL24*, *SVP* or *SOC1*. The repression of *AGL24* by induced *LFY* activity could be mediated through certain unknown mediator(s) (Yu et al., 2004). Moreover, *SVP* and *SOC1* are not upregulated in *lfy* FMs (Gregis et al., 2008; Liu et al., 2007). As *LFY* directly upregulates *AP1* in FMs, it is possible that *LFY* specifies FMs partly through *AP1* (William et al., 2004).

Similar to *ap1* mutants, secondary flowers have also been observed in *Arabidopsis* plants that carry mutations in three of the *SEPALLATA* (*SEP*) floral identity genes, *SEP1*, *SEP2* and *SEP3* (Fig. 5), and, at a higher frequency, in *sep1 sep2 sep3 sep4* quadruple mutants. This indicates that, in addition to their role in specifying floral identity, *SEP1*, *SEP2*, *SEP3* and *SEP4* are also involved in FM specification (Ditta et al., 2004). Both *AGL24* and *SVP* are expressed

in the ectopic FMs of *sep1 sep2 sep3* mutants. Chromatin immunoprecipitation (ChIP) results have further demonstrated the direct binding of SEP3 to *AGL24* and *SVP* promoters, which indicates that SEP3 is involved in directly repressing *AGL24* and *SVP* in FMs (Gregis et al., 2008). *SEP1*, *SEP2* and *SEP4* are expressed throughout stage 2 FMs (Ditta et al., 2004; Flanagan and Ma, 1994; Savidge et al., 1995); this expression pattern overlaps with *API* expression. Although *SEP3* transcripts start to accumulate in the upper portion of late stage 2 FMs (Mandel and Yanofsky, 1998), protein localization analysis has recently shown the presence of SEP3 protein in FMs from stage 1 onwards (Urbanus et al., 2009). Furthermore, AP1 interacts with SEP proteins (except SEP2) in yeast (de Folter et al., 2005). These results indicate that AP1 and SEPs might form protein complexes to maintain FM identity by directly suppressing the expression of *AGL24* and *SVP*. In addition, ectopic *AGL24* and *SVP* expression is also detectable in mutants

with loss-of-function mutations in the floral identity gene *AGAMOUS* (*AG*), which display defects in FM termination and in the growth of reproductive organs (Gregis et al., 2008; Lenhard et al., 2001; Mizukami and Ma, 1997). Therefore, the precise control of *AGL24* and *SVP* expression seems to be a consistent mechanism that is required for FM specification and flower development (Fig. 5).

However, the role played by *AGL24* and *SVP* in FMs is still unclear. Based on the alleviation of *ap1* floral phenotypes by *agl24* or *svp*, it has been proposed that, in the absence of *API*, *AGL24* or *SVP* might recapture its function during the floral transition to promote either inflorescence or vegetative shoot identity in FMs, respectively (Liu et al., 2007; Yu et al., 2004). The observation that FMs are transformed into IMs or shoot meristems upon overexpression of *AGL24* or *SVP* supports this possibility. By contrast, based on the FM-to-IM transition that is observed in *ap1*

Table 1. Members of *StMADS11*-clade MADS-box genes that affect floral meristem development

Plant species	Gene name	Floral phenotypes of gene overexpression	Other functions	References
<i>Arabidopsis thaliana</i>	<i>SHORT VEGETATIVE PHASE</i> (<i>SVP</i>)	Transformation of flowers into shoot-like structures with chimaeric characteristics of vegetative shoots and flowers, loss of carpels (in <i>Arabidopsis</i>).	Repression of flowering (in <i>Arabidopsis</i>).	Hartmann et al., 2000; Liu et al., 2007; Masiero et al., 2004
	<i>AGAMOUS-LIKE 24</i> (<i>AGL24</i>)	Leaf-like sepals and petals, secondary inflorescences in axils of sepals, transformation of carpels into inflorescences (in <i>Arabidopsis</i>).	Promotion of flowering (in <i>Arabidopsis</i>).	Yu et al., 2002; Yu et al., 2004
<i>Antirrhinum majus</i>	<i>INCOMPOSITA</i> (<i>INCO</i>)	Flowers with leaf-like structures, branched trichomes on sepals, petals and carpels, initiation of secondary inflorescences within the gynoecium (in <i>Arabidopsis</i>).	Repression of prophyll development (in <i>Antirrhinum</i>); repression of flowering (in <i>Arabidopsis</i>).	Masiero et al., 2004
<i>Brassica campestris</i>	<i>BcSVP</i>	Pale green petals, elongation of the carpel, alteration in floral organ number (in <i>Arabidopsis</i>).	Repression of flowering (in <i>Arabidopsis</i>).	Lee et al., 2007a
<i>Eucalyptus grandis</i>	<i>Eucalyptus grandis svp</i> (<i>EgrSVP</i>)	Leaf-like perianth organs with increased number of trichomes, indeterminate flower, multiple inflorescences (in <i>Arabidopsis</i>).	Slight repression of flowering (in <i>Arabidopsis</i>).	Brill and Watson, 2004
<i>Hordeum vulgare</i>	Barley <i>MADS1</i> (<i>BM1</i>)	Leaf-like sepals and petals, inflorescences within flowers (in <i>Arabidopsis</i>); inhibited spike development, floral reversion with florets replaced by inflorescence-like structures (in barley).	Unknown	Trevaskis et al., 2007
	Barley <i>MADS10</i> (<i>BM10</i>)	Leaf-like sepals and petals, inflorescences within flowers (in <i>Arabidopsis</i>); inhibited spike development, floral reversion with florets replaced by inflorescence-like structures (in barley).	Unknown	Trevaskis et al., 2007
<i>Lolium perenne</i>	<i>LpMADS10</i>	Enlarged leaf-like sepals and small narrow greenish petals in <i>svp41</i> (in <i>Arabidopsis</i>).	Unknown	Petersen et al., 2006
<i>Oryza sativa</i>	<i>OsMADS22</i>	Occasional secondary flowers in axils of leaf-like sepals; trichomes on sepals (in <i>Arabidopsis</i>); aberrant floral morphogenesis, such as undeveloped paleas and elongated glumes (in rice).	Repression of brassinosteroid responses (in rice).	Fornara et al., 2008; Lee et al., 2008b; Sentoku et al., 2005
	<i>OsMADS55</i>	Abnormal florets (in rice).	Repression of brassinosteroid responses (in rice).	Lee et al., 2008b
	<i>OsMADS47</i>	Occasional secondary flowers in axils of leaf-like sepals; trichomes on sepals (in <i>Arabidopsis</i>).	Repression of brassinosteroid responses (in rice).	Duan et al., 2006; Fornara et al., 2008

agl24 svp triple mutants (Gregis et al., 2008), *AGL24* and *SVP* have been suggested to promote FM fate. Although the function of *AGL24* and *SVP* in FM specification needs to be elucidated further, the current consensus appears to be that, overall, these two factors promote FM indeterminacy (Fig. 5).

Homologues of *AGL24* and *SVP*

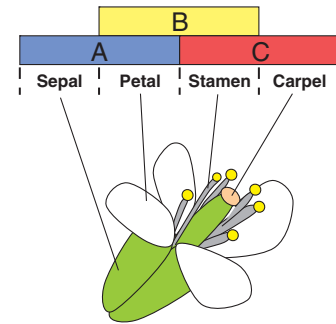
AGL24 and *SVP* are members of the *StMADS11* clade of MADS-box genes (Becker and Theissen, 2003). Recent studies have identified members of this clade in a wide range of plant species. Notably, the overexpression of many *StMADS11*-like genes from dicotyledons and monocotyledons in *Arabidopsis* results in floral phenotypes similar to those produced by the transgenic expression of *35S:SVP* or *35:AGL24* (Table 1), which indicates that these genes could share common functional properties in FM development. In *Antirrhinum*, the *StMADS11* member *INCOMPOSITA* (*INCO*) acts with *FLO* and *SQUA* to specify FM identity (Masiero et al., 2004). Interestingly, *INCO* has been found to either inhibit or promote FM identity in different *Antirrhinum* mutant backgrounds (Masiero et al., 2004). As *squa inco* double mutants produce more flowers than do *squa*, *INCO* appears to prevent the development of reproductive axillary meristems into flowers in this context. Furthermore, the overexpression of *INCO* in *Arabidopsis* produces flowers with vegetative characters that are similar to the flowers of *35S:SVP* transgenic plants. These results suggest that *INCO* represses FM identity. By contrast, in *Antirrhinum flo-662 inco* double mutants, *inco* enhances the FM defect shown in the weak *flo-662* mutant, with axillary inflorescences being generated instead of flowers, which suggests that *INCO* and *FLO* act together to promote FM identity. These contradictory functions of *INCO* in the regulation of FM identity could be due to the interaction of *INCO* with additional protein partners. It has been suggested that, in the presence of *SQUA*, the *INCO-SQUA* heterodimer might act together with *FLO* to specify FM identity whereas, in the absence of *SQUA*, the *INCO* homodimer might inhibit FM identity (Masiero et al., 2004). It is noteworthy that protein-protein interactions between AP1 homologues (e.g. *SQUA*) and *StMADS11*-clade regulators (e.g. *INCO*) have been detected in several plant species, such as *Arabidopsis* (de Folter et al., 2005), *Pharbitis nil* (Kikuchi et al., 2008) and wheat (*Triticum aestivum* L.) (Kane et al., 2005). Thus, it will be important to investigate whether the function of *StMADS11*-like genes in regulating FM identity is modulated through protein interactions with additional FM identity genes, particularly with members of the AP1/*SQUA* gene clade.

In monocotyledons, *StMADS11*-like genes also affect FM identity. The overexpression of barley *MADS1* (*BM1*) and *BM10* inhibits floral development and results in floral reversion in both barley and *Arabidopsis* (Trevaskis et al., 2007). In addition, the overexpression of *Oryza sativa MADS22* (*OsMADS22*) or *OsMADS47* in *Arabidopsis* causes floral reversion and floral defects that are similar to the phenotypes observed when overexpressing *SVP* or *AGL24* (Fornara et al., 2008). These results indicate that *StMADS11*-clade genes might play conserved roles in regulating FM identity. Although the mechanisms of action of these genes still need to be investigated further, the appropriate control of their expression in FMs seems to be crucial for the maintenance of FMs, which lays the foundation for further normal floral patterning.

Repression of floral homeotic genes

Another key aspect in the maintenance of FM identity is the prevention of precocious differentiation triggered by the onset of expression of floral homeotic genes that specify floral organ identity.

Box 2. The ABC model of floral organ development in *Arabidopsis*



According to the ABC model of *Arabidopsis* floral organ development, each whorl of floral organs is determined by the combinatorial action of the class A, class B and class C floral homeotic genes. Class A floral homeotic genes specify sepals in the first whorl. A combination of class A and class B genes specifies petals in the second whorl, whereas a combination of class B and class C genes specifies stamens in the third whorl. Carpel identity in the fourth whorl is determined by the class C gene alone.

In *Arabidopsis*, each whorl of floral organs is determined by the combinatorial action of the class A, class B and class C floral homeotic genes (Box 2) (Bowman et al., 1991; Coen and Meyerowitz, 1991). Three out of four *Arabidopsis* floral organ identities, namely petals (see Glossary, Box 1), stamens and carpels, are controlled by class B and/or class C homeotic genes, which are only activated in early stage 3 FMs. In many mutants in which class B or class C genes are precociously activated in a deregulated pattern, FMs prior to stage 3 that contain insufficient numbers of meristem cells are compelled to enter the floral organogenesis program, which results in a reduced number of floral organs and in the deregulation of floral organ identities. It appears that the repression of floral homeotic genes is the 'default' programme in emerging FMs to ensure that floral anlagen fully develop into stage 3 FMs that contain sufficient cells for the proper patterning of whorled organs by floral homeotic genes. During the past few years, efforts have been made to understand how floral homeotic genes are appropriately repressed in emerging FMs (Table 2).

Transcriptional regulators

SEUSS (*SEU*) and *LEUNIG* (*LUG*) are transcriptional co-regulators that negatively regulate the expression of the class C floral homeotic gene *AG* (Franks et al., 2002; Liu and Meyerowitz, 1995). Flowers of *Arabidopsis seu lug* double mutants exhibit severe floral homeotic transformation, with ectopic *AG* expression throughout the FMs. *SEU* interacts with *LUG* to form a protein complex (Sridhar et al., 2004), and ChIP assays have shown that this *SEU-LUG* complex directly associates with the *AG* promoter (Sridhar et al., 2006). Because neither *SEU* nor *LUG* contains a DNA-binding domain, an interesting question is how they are directed to the promoters of their target genes. AP1 has been identified as an interacting partner of *SEU* (Sridhar et al., 2006). Comprehensive yeast two-hybrid assays among *Arabidopsis* MADS-box proteins show that AP1 can also interact with *AGL24* or *SVP* (de Folter et al., 2005). Moreover, the *LUG-SEU* co-repressor complex interacts with AP1-*AGL24* and AP1-*SVP* dimers (Gregis et al., 2006), which

indicates that these proteins might form a higher-order protein complex to control target gene expression. The observations that *agl24 svp ap1* triple mutants show *lug*-like floral defects and that *AG* is ectopically expressed in FMs of *agl24 svp* double mutants support a common role for *AP1*, *AGL24* and *SVP* in preventing the ectopic expression of *AG* in FMs (Gregis et al., 2006).

Recently, it has been shown that the expression of class B and class C homeotic genes in FMs before stage 3 is redundantly repressed by *AGL24*, *SVP* and *SOC1* through the direct repression of *SEP3* (Liu et al., 2009). In *soc1 agl24 svp* triple mutants, strong ectopic *SEP3* activity interacts with *LFY* activity to synergistically activate class B and class C floral homeotic genes in floral anlagen and emerging FMs, resulting in striking floral defects, such as the loss of most floral organs and the generation of chimeric floral structures (Liu et al., 2009). Thus, *AGL24*, *SVP* and *SOC1* suppress *SEP3* to regulate the timing of floral organ patterning by inhibiting the ectopic expression of floral homeotic genes in young FMs. These results suggest that the regulation of *AGL24*, *SVP* and *SOC1* at an appropriate level is crucial for the

maintenance of FMs because their elevated expression causes FM indeterminacy, whereas lower expression causes the precocious differentiation of FMs (Fig. 5).

The activation of the class C gene *AG* is required for the termination of FMs through the repression of a meristem gene, *WUSCHEL* (*WUS*), which is necessary for maintaining FMs in a proliferative and indeterminate state (Lenhard et al., 2001; Lohmann et al., 2001); this repression was recently found to be mediated by the C2H2-type zinc finger protein *KNUCKLES* (Sun et al., 2009). *LFY* and *WUS* act together to induce *AG* expression in FMs, and the participation of *SEP3* in *AG* induction implies that the direct regulators of *SEP3*, namely *AGL24*, *SVP* and *SOC1*, play a role in preventing FM termination through mediation of the timing of *AG* expression (Fig. 5).

Chromatin modulators

Another important group of regulators involved in repressing floral homeotic gene expression are chromatin regulators, many of which are Polycomb Group (PcG) proteins that affect chromatin states to

Table 2. *Arabidopsis* genes that prevent precocious activation of floral homeotic genes

Gene name	Gene identity	Expression in FMs	Floral homeotic genes derepressed in mutants	Mutant tissues where floral homeotic genes are derepressed	Association with floral homeotic gene promoters
<i>BELLRINGER (BLR)</i>	Homeobox protein	Yes (Bao et al., 2004)	<i>AG</i> (Bao et al., 2004)	Inflorescence apices	Unknown
<i>CURLY LEAF (CLF)</i>	E(z) orthologue; a PRC2 component	Yes (Goodrich et al., 1997)	<i>AG</i> , <i>AP3</i> (Goodrich et al., 1997)	Vegetative tissues (<i>AG</i> and <i>AP3</i>); inflorescence stems (<i>AG</i>)	Yes (<i>AG</i>) (Schubert et al., 2006)
<i>EMBRYONIC FLOWER 1 (EMF1)</i>	Plant specific repressor; proposed to play a PRC1-like role	Unknown	<i>AP3</i> , <i>PI</i> , <i>AG</i> (Moon et al., 2003)	Vegetative tissues	Yes (Calonje et al., 2008)
<i>EMBRYONIC FLOWER 2 (EMF2)</i>	Su(z)12 orthologue; a PRC2 component	Yes (Yoshida et al., 2001)	<i>AP3</i> , <i>AG</i> (Chanvivattana et al., 2004)	Vegetative tissues	Unknown
<i>FERTILIZATION-INDEPENDENT ENDOSPERM (FIE)</i>	Esc orthologue; a PRC2 component	Unknown	<i>AP3</i> , <i>AG</i> (Katz et al., 2004)	Vegetative tissues	Unknown
<i>INCURVATA2 (ICU2)</i>	Catalytic subunit of the DNA polymerase α	Unknown	<i>AP1</i> , <i>AP3</i> , <i>PI</i> , <i>AG</i> , <i>SEP3</i> (Barrero et al., 2007)	Vegetative tissues	Unknown
<i>LEUNIG (LUG)</i>	Co-repressor	Yes (Conner and Liu, 2000)	<i>AP3</i> , <i>PI</i> , <i>AG</i> (Liu and Meyerowitz, 1995)	Sepals (<i>AP3</i> , <i>PI</i> and <i>AG</i>); petals (<i>AG</i>)	Unknown
<i>MULTICOPY SUPPRESSOR OF IRA1 (MSI1)</i>	p53 orthologue; a PRC2 component	Unknown	<i>AG</i> (Hennig et al., 2003)	Vegetative tissues	Unknown
<i>ROXY1</i>	Glutaredoxin	Yes (Xing et al., 2005)	<i>AG</i> (Xing et al., 2005)	Stage 2 FMs (only found in <i>roxy1 ap1</i> double mutants)	Unknown
<i>SEUSS (SEU)</i>	Plant specific regulatory protein	Yes (Azhakanandam et al., 2008)	<i>AG</i> (Franks et al., 2002)	Stage 2 FMs	Yes (Sridhar et al., 2006)
<i>SWINGER (SWN)</i>	E(z) orthologue; a PRC2 component	Yes (Chanvivattana et al., 2004)	Unknown	Unknown	Unknown
<i>TERMINAL FLOWER 2 (TFL2; LIKE HETEROCHROMATIN PROTEIN 1, LHP1)</i>	HP1 homologue	Yes (Kotake et al., 2003)	<i>AP1</i> , <i>AP3</i> , <i>PI</i> , <i>AG</i> , <i>SEP3</i> (Kotake et al., 2003)	Vegetative tissues	Yes (Zhang et al., 2007)

inhibit the transcription of floral homeotic genes (Table 2). The PcG proteins EMBRYONIC FLOWER 2 (EMF2), CURLY LEAF (CLF), SWINGER (SWN), FERTILIZATION-INDEPENDENT ENDOSPERM 1 (FIE1) and MULTICOPY SUPPRESSOR OF IRA1 (MSI1) form a putative Polycomb Repressive Complex 2 (PRC2) that catalyzes the tri-methylation of lysine 27 of histone H3 (H3K27me3) of target genes, leading to their transcriptional silencing (Chanvattana et al., 2004; Farrona et al., 2008; Goodrich et al., 1997; Hennig et al., 2003; Katz et al., 2004). Mutations in *clf*, *emf2*, *fie* or *msi1* cause the ectopic expression of floral homeotic genes, even in embryos or vegetative seedlings, indicating that these PRC2 components are required for the repression of floral homeotic gene expression during plant development. EMBRYONIC FLOWER 1 (EMF1), a potential PRC1-like factor that maintains the transcriptional repression of targets by recognizing H3K27me3, acts together with the EMF2 complex to repress *AG* expression during vegetative development (Calonje et al., 2008).

TERMINAL FLOWER 2 (TFL2; also known as LIKE HETEROCHROMATIN PROTEIN 1, LHP1) is probably also an *Arabidopsis* PRC1-like factor and is homologous to Heterochromatin Protein 1 (HP1) in metazoans and yeast, a protein that plays important roles in chromatin packaging and gene silencing (Gaudin et al., 2001; Kotake et al., 2003). *TFL2* is expressed in proliferating cells, including those of FMs, and the encoded TFL2 protein preferentially binds to chromatin marked with H3K27me3 in vivo (Zhang et al., 2007). TFL2 is directly associated with the regulatory sequences of a group of floral homeotic genes, such as *AP3*, *PISTILLATA (PI)*, *AG* and *SEP3*, and suppresses their expression during vegetative growth (Kotake et al., 2003; Turck et al., 2007; Zhang et al., 2007). The protein interaction between TFL2 and INCURVATA2, a DNA polymerase subunit probably involved in DNA replication (Barrero et al., 2007), indicates a role for the replication machinery in the maintenance of gene silencing.

It is noteworthy that almost all of the above-mentioned chromatin regulators are ubiquitously expressed in *Arabidopsis*. Thus, an important question is how they are specifically regulated to permit the onset of floral homeotic gene expression in FMs at stage 3. A recent study has revealed that the orchestrated repression of *SEP3* by SVP, AGL24 and SOC1 is mediated by recruiting two interacting chromatin regulators, TFL2 and SAP18, a member of the SIN3 histone deacetylase complex (Liu et al., 2009). The downregulation of *AGL24*, *SVP* and *SOC1* in FMs disrupts the histone-modification function of TFL2 and SAP18 at the *SEP3* locus, thus derepressing *SEP3*, which in turn contributes to the activation of other floral homeotic genes such as *AP3*, *PI* and *AG*. This finding suggests that the developmental specificity of chromatin regulators could be achieved by regulating the levels of their interacting transcription factors.

Conclusions

Over the past few years, an ever-expanding list of regulators has emerged that form regulatory hierarchies to govern the successive developmental programmes involved in FM specification. The translation of flowering signals into actual flower formation requires the coordinated control of these regulatory hierarchies by certain common factors. In *Arabidopsis*, the FM identity genes *API* and *LFY*, and the flowering-time genes *AGL24*, *SVP* and *SOC1*, participate in this control by linking the molecular events that regulate their activity with the regulation of their downstream targets. Investigating the homologues of these *Arabidopsis* genes in other plant species has unravelled the functional conservation and divergence of their counterparts in governing the specification of

FMs. These advances in understanding FM specification have, however, raised some additional questions to which answers are still outstanding. For example, although we know that auxin contributes to FM initiation, it is unclear how flowering-time genes affect the auxin pathway to trigger FM formation, and how the auxin pathway interacts with known FM regulators, such as *LFY* and *API*, to specify FM identity. In addition, the biological significance of FM polarity regulation remains to be elucidated. Addressing these questions by comprehensive molecular, genetic and biochemical approaches will greatly contribute to our understanding of the combinatorial control of FM specification.

Note added in proof

Two recent studies (Wang et al., 2009; Yamaguchi et al., 2009) provide evidence for the involvement of miRNA-regulated SQUAMOSA PROMOTER BINDING PROTEIN-LIKE transcription factors in the regulation of flowering.

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