Specification and maintenance of the floral meristem: interactions between positively-acting promoters of flowering and negative regulators

Usha Vijayraghavan[1,*], Kalika Prasad[1] and Elliot Meyerowitz[2,*]

[1]Department of MCB, Indian Institute of Science, Bangalore 560 012, India
[2]Division of Biology 156–29, California Institute of Technology, Pasadena, CA 91125, USA

A combination of environmental factors and endogenous cues trigger floral meristem initiation on the flanks of the shoot meristem. A plethora of regulatory genes have been implicated in this process. They function either as activators or as repressors of floral initiation. This review describes the mode of their action in a regulatory network that ensures the correct temporal and spatial control of floral meristem specification, its maintenance and determinate development.

Keywords: *Arabidopsis thaliana*, floral activators, floral mesitem specification, floral repressors.

THE angiosperm embryo has a well established apical-basal/polar axis defined by the positions of the root and shoot meristems. Besides this, a basic radial pattern is also established during embryogenesis. This embryonic shoot apical meristem (SAM) is the progenitor of organs and organ systems that form all above ground portions of adult land plants. Genetically defined regulators of organ patterning are best understood in *Arabidopsis thaliana*, a laboratory model plant of the mustard family that is amenable to molecular genetic studies. These studies provide a detailed framework to examine both evolutionarily conserved and species-specific aspects of organ patterning in other plants. In *Arabidopsis*, as in other flowering plants, the SAM can be subdivided into layers and zones (Figure 1 *a*)[1]. The central zone (CZ) of the SAM contains infrequently dividing stem cells at the top. The displaced daughter cells from the CZ contribute to peripheral zone (PZ) where their frequent yet regulated proliferation produces lateral organ primordia or lateral meristems. Below the organizing centre of CZ is the rib zone whose progeny form the central tissues of the shoot axis. Thus shoot meristems perform two functions: (i) they produce cells for lateral organ primordia or lateral meristems and for differentiated tissues of stem; (ii) they maintain the stem cell pool throughout the life of the plant. Flowers are produced from floral meristems, specialized lateral shoot meristems that give rise to modified leaves – whorls of sterile organs (sepals and petals) and reproductive organs (stamens and carpels). In this review we focus on mechanisms by which interactions between positive and negative regulators together pattern floral meristems in the model eudicot species *A. thaliana*.

*For correspondence. (e-mail: uvr@mcbl.iisc.ernet.in; meyeerow@caltech.edu)

Maintenance of the shoot apical meristem and transitions in lateral meristem fate

The maintenance of stem cells is brought about, at least in part, by a regulatory feedback loop between the homeodomain transcription factor *WUSCHEL* (*WUS*) and genes of the *CLAVATA* (*CLV*) signaling pathway[2]. *WUS* is expressed in the organizing centre and confers a stem cell fate to overlying cells. These stem cells then express CLV3, the peptide ligand, for the CLV1 receptor a serine/threonine kinase. An unknown signal activated when CLV3 binds to CLV1. This represses *WUS* expression closing the feedback loop. Reduced *WUS* expression results in fewer stem cells, and thus in turn less repressive CLV3–CLV1 interaction (Figure 1 *b*). This regulatory mechanism maintains the stem cell pool throughout the plant's life. Recent studies indicate a short 57 bp cis-acting element in *WUS* promoter mediates the effects of diverse regulatory pathways controlling *WUS* expression[3]. Lateral organ formation initiates from the PZ of the shoot meristem where a group of cells derived from all three meristem layers (L1, L2 and L3) are assigned to an incipient organ primordium[4]. In order to initiate lateral organ primordia within the PZ, the expression of another homeodomain transcription factor *SHOOT MERISTEMLESS* (*STM*) has to be down-regulated in the organ founder cells[5]. The expression of *STM* throughout the SAM but not in the organ founder cells[5] prevents the apical meristem dome from premature differentiation by repressing the leaf primordium-specific regulator *ASYMMETRIC LEAVES1* (*AS1*) (Figure 1 *b*)[6]. How the initial down-regulation of *STM* takes place at the sites of organ initiation remains unknown.

In *Arabidopsis*, upon induction of flowering, the shoot meristem turns into an inflorescence meristem which produces floral meristems (Figure 1 *c*), instead of leaf primordia, on its flanks. Genes involved in specification and development of floral meristem can be generally categorized into two groups: first, those that specify the young flower and second a group of genes that prevent the shoot from precociously adopting a floral fate[7].

Chromatin regulators controlling floral meristem specification

Many chromatin regulators such as *EMBRYONIC FLOWER2* (*EMF2*), *SPLAYED* (*SYD*), *TERMINAL FLOWER2* (*TFL2*), *atISWI*, *FIE* and *CURLY LEAF* (*CLF*) affect floral initiation by acting as flowering repressors during the vegetative phase of growth[8]. Factors like EMF2 and FIE repress expression, during vegetative phase, of genes that specify floral meristems like *LEAFY* (*LFY*) and *APETALA1* (*AP1*). EMF2, FIE and CLF form components of at least one subtype of Polycomb complex that repress floral organ identity MADS-box genes[9] and thus prevent floral organogenesis in vegetative tissues (Figure 2).

Maintenance of the shoot apical meristem requires one of four members of *Arabidopsis* class SNF2 ATPases SYD[10]. Genetic analysis of SAM defects in double mutants of *syd* combined with mutants in other meristem regulating factors indicates that *SYD* largely acts in the *WUS* pathway[11]. Furthermore, this study found that SYD regulates the stem cell pool in the SAM via direct transcriptional control of *WUS*, a central regulator of SAM maintenance (Figure 2). SYD is required for up-regulation of *WUS* transcription and it binds a proximal promoter region in the *WUS* locus[11]. Besides this role in maintenance of stem cells, SYD also influences meristem identity. It acts as repressor of LFY-dependent activity prior to floral transition. But after the floral transition, *SYD* acts as a redundant *LFY* co-activator for the induction of the class B and class C floral organ patterning genes (Figure 2)[10]. Early flowering of *syd* mutants in non-inductive short-days (SD) suggests the repressive activity of SYD, to certain extent, is photoperiod sensitive. Thus, *SYD* provides a unique example of chromatin remodelling factor that links an environmental signal to a key floral meristem identity molecule for repression of the floral meristem and thus for its proper development.

Function of MADS-box genes as repressors of floral meristem formation

In addition to chromatin regulators, MADS-domain transcription factors are also involved in maintaining the shoot meristem by repressing floral initiation. An *Arabidopsis* MADS-box gene *SHORT VEGETATIVE PHASE* (*SVP*) functions as a repressor of the floral transition. *svp* mutants flower earlier than the wild type[12]; whereas *SVP* ectopic over-expression dramatically delays floral transition[13]. Consistent with its regulatory role in meristem maintenance, *SVP* is expressed throughout the SAM during vegetative development. After the floral transition it is expressed[12] in young flower primordia until stage 3. SVP perhaps affects the activity of positive regulators of floral meristem identity such as AP1, CAULIFLOWER (CAL), SEPALLATA1 (SEP1) and SEP2 – with whom physical interactions are detected by the yeast two-hybrid screens[13,14].

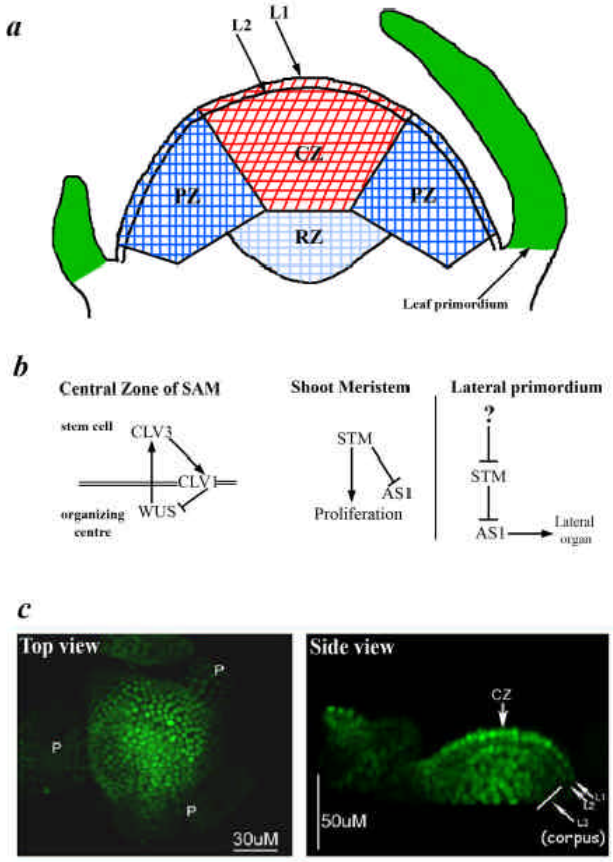

Figure 1. Organization of shoot meristem and regulatory pathways that control its maintenance. ***a***, Schematic diagram of the *Arabidopsis* shoot apical meristem (SAM) with developing lateral organs. The SAM is organized in layers and zones. The infrequently dividing central zone (CZ) contains organizing centre with overlying stem cells. Frequently dividing cells in the peripheral zone (PZ) give rise to lateral organs, whereas divisions below the rib zone (RZ) contributes to growth of the shoot axis. ***b***, Regulatory pathways active in the shoot meristem. Shoot stem cells are maintained by the *WUS–CLV* feedback loop. *WUS* expression in organizing centre confers stem cell identity. The CLV3 ligand secreted by stem cells is thought to bind to the receptor CLV1 which in turn represses *WUS* expression (denoted by T-bar). STM maintains proliferation in shoot meristem by repressing expression of *AS1*. *STM* is repressed (denoted by T-bar) in lateral primordia permitting activation of *AS1* expression that is required for lateral organ development. ***c***, Confocal laser scanning micrograph of an inflorescence meristem with emerging young floral primordia (P). Nuclei in all cells are marked by the expression of histone2B::GFP.

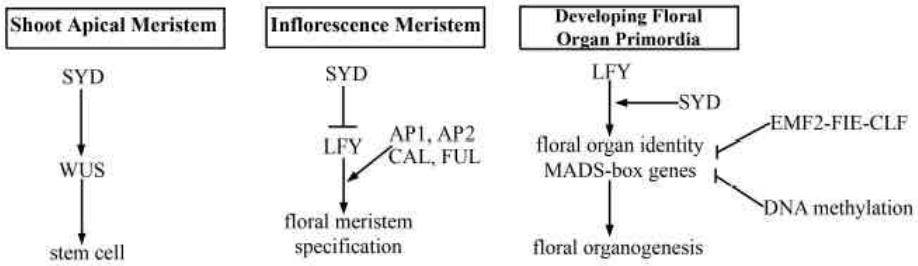

Figure 2. Chromatin regulators affecting meristem specification. The chromatin modifier SYD directly activates *WUS* to maintain stem cells in the shoot meristem, while it represses the *LFY* dependent activity in the inflorescence meristem. Redundant activities of floral meristem identity genes *LFY*, *AP1*, *CAL*, *AP2* and *FUL* specify floral meristems. SYD functions with *LFY* to activate floral organ identity genes whose expression in vegetative tissues is repressed by polycomb group chromatin repressive EMF2–FIE–CLF complex. Activation and repression are denoted by arrows and T-bars, respectively.

Thus interactions between positive and negative regulators critically influence specification of the floral meristem. Interestingly, *Antirrhinum SVP* homologue *INCOMPOSITA* (*INCO*) functions as a positive and a negative regulator of floral meristem specification[13].

Similarly, another *Arabidopsis* MADS-domain factor *AGL24*, closely related to *SVP*, represses floral meristem specification since it promotes an inflorescence fate. *AGL24* is expressed throughout the shoot and inflorescence meristem but its expression in the floral meristem is limited to a single cell layer[15]. Floral meristem-promoting factors *LFY* and *AP1* repress *AGL24* since the inflorescence characteristics of *lfy* and *ap1* mutant flowers are seen mainly due to the continued ectopic expression of *AGL24* in these mutant shoot-like floral meristems[15].

A balance between floral repressors and floral activators fine-tune floral meristem specification

The activity of key floral meristem identity genes *LFY* and *AP1* is further repressed by *TERMINAL FLOWER1* (*TFL1*) in inflorescence meristem (Figure 3). The *Arabidopsis terminal flower1* (*tfl1*) mutant terminates its apical meristem after the initial production of a few lateral flowers. This suggests that while the inflorescence meristem is established, its maintenance fails resulting in its conversion to floral meristems[16]. In fact the early flowering phenotypes observed upon ectopic overexpression of the floral meristem identity genes *LFY* or *AP1*[17,18] may arise from repression of *TFL1* since these genes have complementary expression patterns and loss-of-function phenotypes[18]. *TFL1* is expressed in the inflorescence meristem, while the floral meristem identity genes are expressed in newly arising floral meristems[19–21]. Analysis of *TFL* expression levels upon over expression of floral meristem activators *LFY* or *AP1* and the converse study of the phenotypic consequences of *TFL* over expression together suggest that *TFL1* inhibits the expression of key floral meristem identity genes *LFY* and *AP1*, and vice versa (Figure 3)[22,23]. *LFY*, *AP1* and *CAL* inhibit *TFL1*

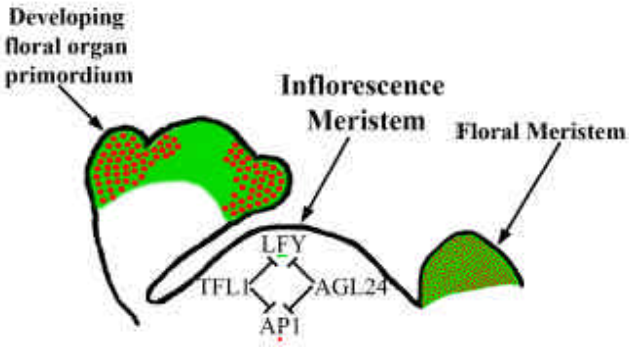

Figure 3. Diagrammatic representation of young floral meristems on the flanks of the inflorescence meristem. *TFL1* and *AGL24* repress (denoted by T-bars) key floral meristem identity genes *LFY* and *AP1* in the inflorescence meristem. Uniform accumulation of *LFY* and *AP1* transcripts in the young stage 2 floral meristem, to the right, is represented by uniform green colour with red dots. At stage 5 when floral organ primordia are being initiated *AP1* expression (red dots) is restricted to the developing first whorl (sepal) and second whorl (petal) primordia while *LFY* expression continues in all floral organ primordia (green zone).

transcriptionally; in contrast, inhibition of expression of floral meristem identity genes by *TFL* occurs in two ways. First, *TFL1* retards up-regulation of these genes by delaying the progression of the reproductive phase. Secondly, *TFL1* prevents a response to *LFY* and *AP1* even when they are expressed at high levels[23]. Ectopic expression of floral repressor *TFL1* in *ap1 cal ful* triple mutants contributes to their non-flowering phenotype suggesting *AP1*, *CAL* and *FUL* act redundantly in specifying the floral meristem at least in part by regulating the expression domain of *TFL1*[24]. TFL belongs to a family of proteins with properties of binding phosphatidylethanolamine (PEBP), similar to FT, a factor involved in floral induction, suggesting functions in signal transduction for both factors[25,26]. Interestingly, despite being similar molecules, mutants in these factors have complementary phenotypes and it is possible that they regulate the same step in flowering. The biochemical functions of *TFL* or *FT* are yet to be demonstrated.

Activation of flowering pathway integrators and floral initiation

Distinct flowering pathways in response to day-length, the phytohormone gibberellic acid (GA), changes in light quality and ambient temperature promote the transition from vegetative to reproductive phase by activating the flowering pathway integrators: *FT* and a MADS-box gene *SUPRESSOR OF CONSTANS1* (*SOC1*)[27]. These flowering pathway integrators function as positive regulators of floral meristem identity genes whose redundant activities in turn specify the floral meristem (Figure 4). The floral meristem promoting effects of long days are largely through the effect of the photoperiod-dependent regulator *CONSTANS* (*CO*), a transcription factor whose action couples the circadian clock and the flowering pathway integrators *FT* and *SOC1*. The photoperiod-regulated accumulation of CO protein occurs by both transcriptional and post-transcriptional control. Light in the later part of the day/night cycle enhances *CO* transcription and also stabilizes the protein[28]. This allows for activation of *FT* and the downstream effects of activation of floral meristem determining factors. Very recent studies elucidate how *CO*-dependent spatial and temporal regulation of floral meristem specification takes place. Photoperiod perception and the *CO* dependent transcriptional up-regulation of *FT* occurs in the leaves and spatial transfer of this information to the shoot apex is necessary to effect a change in the identity of the emerging lateral meristems. This is achieved, at least in part, by movement of the *FT* RNA to the shoot apex perhaps in conjunction with other signals[29]. At the shoot apex interactions between *FT* and *FD*, a b-HLH domain containing transcription factor contributes to activation of floral meristem determinant *AP1* in the emerging lateral meristems[30,31]. How this pathway of *FT-FD* based activation of *AP1* interacts with other positively and negatively acting factors that also contribute to floral meristem specification is yet to be explored.

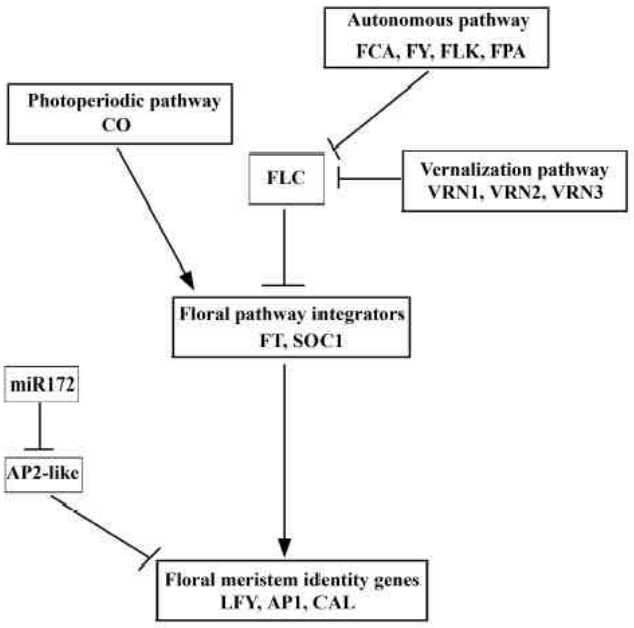

Figure 4. Schematic representation of the pathways affecting floral initiation in *Arabidopsis thaliana*. The photoperiodic pathway promotes the activity of flowering pathways integrators *FT* and *SOC1*. Repression of *FLC* by components of autonomous and vernalization pathways allows accumulation of floral integrators which in turn activate floral meristem identity genes. Activation of floral meristem identity genes is also regulated by a microRNA (miRNA) pathway, with the miRNA based repression of *AP2*-like factors being shown here. Activating and repressive functions are denoted by arrows and T-bars, respectively.

Accumulation of transcripts for flowering pathways integrators and thus floral meristem identity genes, a prerequisite for floral meristem specification and initiation, also requires the repression of a floral repressor *FLC*, a MADS-box gene (Figure 4). *FLC* expression is controlled by both post-transcriptional and chromatin modification mechanisms. *FLC* repression at the chromatin level requires *HUA ENHANCER1-1* (*HEN1-1*) where *HEN1* is involved in the production of a siRNA homologous to *FLC* intron1. These siRNAs trigger chromatin remodelling within intron1 of the *FLC* locus, by dimethylation of histone H3, leading to silencing of *FLC* expression[32]. The post-transcriptional regulation of *FLC* expression occurs through *FCA* – a nuclear protein containing two RNA recognition motifs (RRM) – an RNA-binding domain and a WW protein interaction domain[33] and FY, a WD-repeat protein. These factors promote premature polyadenylation and thus contribute to repression of active *FCA* expression[34,35].

Additionally these flowering pathway integrators control specification of floral meristems by acting in conjunction with floral meristem identity genes. Single mutants in *SOC1* do not alter floral initiation as evident from their nearly negligible effects on the number of co-inflorescences in the *soc1* mutant[36]. However, when combined with floral meristem identity mutant *lfy*, i.e. in the *soc1 lfy* double mutant, a severe co-inflorescence phenotype is seen with a continuous production of secondary shoot-like structures in addition to the failure to produce any mutant flowers typical of *lfy*. Similarly, double mutants of another flowering pathway integrator *FLOWERING LOCUS T* (*FT*) and *LFY*, i.e. *ft lfy* show a dramatic suppression of floral meristem initiation[36]. Interestingly, *FT* and *LFY* share overlapping functions with regard to activation of *AP1* expression[37]. These studies suggested that integrators in the flowering induction pathway act in parallel with floral meristem specification factors to affect meristem initiation, in addition to their role in flowering time.

Effect of light and hormone-mediated signals in maintaining meristem identity

Phytochrome-mediated pathway and hormone signal transduction pathways besides acting through flowering

pathway integrators also control the establishment of the floral meristem and its determinacy by regulating the activity of floral meristem identity genes. The effect of these signals has been studied genetically using floral mutants *ap2*, *ap1*, *lfy* and *ag*[38–40]. Flowers of *ap2-1* or *ap1* mutant plants grown in short-days (SD) show enhanced inflorescence-like characteristics[41,42]. These enhanced floral phenotypes caused in short-days are due in part to *SPY* gene activity. The *spy-2* mutant suppresses axillary flower development in *ap2-1* flowers grown under SD photoperiod; while the *spy-3* mutant suppresses the strong floral meristem defects of the strong *ap1-1* flowers under both LD and SD conditions[40]. These inflorescence-like characteristics are strongly suppressed by exogenously applied GAs[40]. Thus, floral meristem determining factors are responsive to endogenous and environmental cues.

Phytochrome and GAs affect maintenance of floral meristems once established as deciphered from analysis of *ag* and in *lfy* mutant flowers in short days[39]. These genetic studies provide a link between GA and phytochrome signal transduction and the floral meristem patterning genes *LFY*, *AP1*, *AP2*, and *AG*. The flower-promotion effects of GA, in short days, occur through activation of *LFY* most likely through the action of GAMYB transcription factors. The continued effects of GA on floral organogenesis occur through promoting the expression of floral organ identity genes by repressing the activity of DELLA-domain containing transcription factors[43].

Yet another plant hormone, which plays a pivotal role in plant meristem and organ primordia development is auxin. This plant signalling molecule besides having role in the initiation and positioning of lateral organs such as leaves[44] and lateral roots[45], has also been implicated in positioning the inflorescence derived lateral organs, i.e. flowers[46]. Auxin-dependent pathways are important in later aspects of floral organ differentiation as shown by recent studies where the loss of auxin responsive transcription factors – ARF6 and ARF8 affects the transition from immature flowers to mature flowers[47]. However, it remains largely unknown how auxin influences key regulatory molecules involved in early aspects of floral meristem specification and floral organ primordia initiation.

Role of microRNAs in floral initiation and floral meristem patterning

Emerging evidence shows the regulatory functions for microRNAs (miRNAs) during the floral transition and floral meristem specification. ARGONAUTE1 (AGO1), an essential factor in miRNA mediated pathways, is required for expression of key floral meristem identity genes *LFY* and *AP1*. This crucial role of *AGO1* in specifying the floral meristem is evident from its loss-of-function mutant phenotypes wherein inflorescences lack floral identity[48]. Furthermore, other studies indicate a role for a GA-regulated microRNA (mRNA159) in controlling floral initiation by regulating *LFY* transcript levels and a role in floral organogenesis[49]. *APETALA2* (*AP2*) together with two *AP2*-like genes *TARGET OF EAT1* (*TOE1*) and *TOE2* are potential targets for regulation by a group of miRNAs derived from a family of *MIR172* precursor genes[50,51]. While *AP2* acts redundantly with other floral meristem identity genes to specify the floral meristem (described in the following section)[52], *TOE1* and *TOE2* act as floral repressors[53], since down regulation of *TOE1* and *TOE2* is required during the floral transition. Loss-of-function phenotypes of *TOE1* and *TOE2* together with the suppression, by miR172 over expression, of the late flowering phenotype created upon *TOE1* over expression indicate these genes to be post-transcriptionally regulated by miR172 (ref. 53). miR172 appears to regulate *AP2* (ref. 54), *TOE1* and *TOE2* (ref. 53) at the level of translation rather than by RNA cleavage. However, very recent studies demonstrate that miR172 can guide cleavage of target plant RNAs, thus unifying the general mechanism of action of plant miRNAs[55]. Consistent with the proposed role in regulation of flowering time, miR172 expression is upregulated during the floral transition with expression continuing in young flowers. The temporal up-regulation of miR172 leads to temporal down-regulation of *TOE1* and *TOE2* and thus relieves their repressive effects on floral meristem specification (Figure 4)[51,53]. A link between the miRNA driven post-transcriptional gene regulation and the flowering pathway integrators is suggested by the observation that at least one miRNA precursor gene *MIR172a-2* is up-regulated and the target *AP2*-like genes are down-regulated after floral induction in manner that is dependent on *CO* and *FT*[51].

Redundant activities of floral meristem identity genes in floral meristem specification

Several *Arabidopsis* genes are required to confer floral identity on newly arising meristems. These include *LEAFY* (*LFY*), *APETALA2* (*AP2*), and three closely related genes *APETALA1* (*AP1*), *CAULIFLOWER* (*CAL*) and *FRUITFULL* (*FUL*)[24,56]. *LFY* is a key integrator of flower-promoting pathways and among the floral meristem identity genes is a predominant factor since *lfy* loss-of-function alleles affect floral meristem fate much more severely than mutations in other genes. *lfy* mutants have increased numbers of secondary inflorescences and have abnormal shoot-like flowers in the place of solitary flowers[20,57]. The partial floral features in the *lfy* shoot-like flowers suggest redundant activities for floral meristem fate determining genes. *apetala1* (*ap1*) mutants produce flowers with branched shoot-like features in that they bear reiterating flowers in the axil of first-whorl bract-like organs. However, these mutant flowers have functional reproductive floral organs, indicating that they are

only partially defective in defining floral meristem identity. The phenotypes of *lfy* and *ap1* single mutants and of *lfy ap1* double mutants indicate that these genes have partially redundant functions. The phenotypically silent *cauliflower* (*cal*) mutants are enhancers of *ap1*; the *ap1 cal* double mutants produce reiterating meristems with poor or no floral organ differentiation[52,58,59]. *FRUITFULL* (*FUL*) is yet another gene that contributes to floral meristem specification; *ful* alleles when combined with *ap1 cal* double mutants cause a non-flowering phenotype with the plants continuously producing only leafy shoots. The lack of *LFY* upregulation in these plants explains this phenotype[24]. These findings demonstrate that *FUL* acts redundantly with *AP1* and *CAL* in specifying the floral meristem by regulating *LFY* expression levels.

apetala2 (*ap2*) mutations also enhance the floral meristem defects of *ap1* and *lfy* mutants and thus *AP2* contributes to floral meristem identity[52,58]. All of these floral meristem-determining factors encode transcription factors. While AP1, CAL and FUL proteins contain the DNA-binding MADS domain, *AP2* encodes a protein containing AP2-DNA binding domain (a member of the EREBP class of transcription factors)[19,60,61]. *LFY* encodes a sequence-specific DNA binding transcription factor unique to the plant kingdom[20]. The high levels of *LFY*, *AP1* and *CAL* expression early in the ontogeny of floral primordium formation and even in the floral anlagen (Figure 3) supports a direct role of these genes in determining a floral fate[19,20,62]. LFY directly regulates the transcription of *AP1* and *CAL*[63,64]. The RNA expression of *AP2* and *FUL* differs from *LFY*, *AP1* and *CAL* in that both *AP2* and *FUL* are also expressed in inflorescence meristems, inflorescence stems and cauline leaves besides the young floral meristem[60,61].

Role of *SEPALLATA* MADS-box genes in maintaining floral meristem identity

The closely related MADS-box genes, *SEPALLATA1/2/3* (*SEP1/2/3*) influence floral meristem identity in addition to their main role as co-factors governing organ fate in the second, third and fourth whorls of the flower. Their role in meristem identity is evident from occasional production of secondary flowers in the sepal axils of *sep1 sep2 sep3* triple mutants[65]. Further, even the *sep3*-1 and *sep3*-2 single mutant plants have axillary flowers at the base of sepals, a phenotype that resembles moderate alleles of *ap1*. Additional evidence comes from the observation of interactions among SEP3, CAL and AP1 proteins and the enhanced early flowering phenotype upon over expression of both SEP3 and AP1 proteins[14]. Further, in addition to promoting flowering ectopic expression of *SEP3* can activate downstream floral organ identity genes *APETALA3* and *AGAMOUS*[66].

Loss of floral meristem identity becomes more pronounced in quadruple mutants of various *sep* alleles combined with *ap1* mutants[67]. Among various combinations *sep1 sep2 sep4* triple mutant combined with *ap1* showed a cauliflower phenotype similar to the *ap1 cal* double mutant, suggesting that SEP proteins are required for *CAL* function. In comparison, *ap1 sep1 sep2* or *ap1 sep3* do not show cauliflower-like characters suggesting that among *SEP* genes *SEP4* plays a greater role in specifying the floral meristem[67]. The increased severity of the floral meristem identity defects seen in *ap1 sep4* or *ap1 cal sep4* mutants illustrates its significant role in controlling floral meristem identity.

A suicidal feedback loop terminates the floral meristem

Like the shoot apical meristem (SAM), the floral meristem harbors a population of stem cells that provide cells for developing floral organ primordia in all four whorls thus generating the organs sepal, petal, stamen and carpel. However, unlike the indeterminate *Arabidopsis* shoot meristem, the floral meristem terminates once all floral organ primordia have been initiated. Termination of floral meristem is brought about by a *WUS–AG* feedback loop[68,69]. The interactions among *LFY*, *WUS* and *AG* in the center of *Arabidopsis* flowers provide a mechanism to explain the differential effects of stem cell regulation in shoot versus the flower. Genetic evidence suggests that induction of *AG* by *WUS* is dependent on *LFY*, implicating *LFY* to be the distinguishing factor for stem cell regulation in flowers. WUS acts cooperatively with LFY to activate *AG* (Figure 5 *a*). Early in the establishment of the floral meristem, WUS together with LFY binds sequences to activate *AG* expression. The AG protein thus expressed in turn represses the *WUS* transcription and terminates the floral meristem (Figure 5 *b*). The *WUS–AG* feedback loop is different from the *WUS–CLV3* loop that maintains the shoot apical meristem in that *WUS–AG* loop functions temporally in the same cell population to transform the indeterminate state of the floral meristem to a determinate one. But in the vegetative apical meristem WUS acts in a population of cells known as the organizing centre with

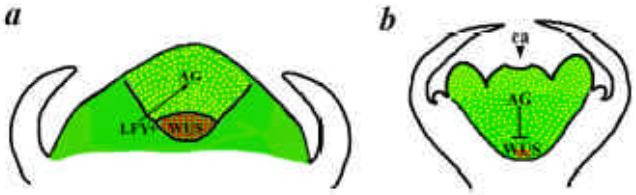

Figure 5. The *WUS–AG* feedback loop controls floral meristem determinacy. ***a***, In a stage 3 floral meristem WUS (denoted by the red hatched area) enhances LFY-mediated expression of *AG* (denoted by yellow dots). ***b***, Enhanced *AG* expression in stage 6 flowers at the time of carpel (ca) initiation terminates stem cell activity by repressing *WUS* expression.

the CLV3 signal emanating from a different set of cells in the apical domain[70].

Conclusions/perspective

Recent studies on homologues of key *Arabidopsis* flowering regulators in evolutionarily divergent grass species have now begun to unravel how these molecules have evolved to retain conserved functions and in instances these studies provide evidence for additional species-specific functions. Investigations on two model plants for grasses – rice and maize – are particularly useful. Studies of the rice *FT* homologue Hd3a and *Hd1*, the homologue for the regulator of *FT*, i.e. *CONSTANS* (*CO*) reveal how evolutionarily conserved factors alter their regulatory capacity to control the same target molecule but distinctly in different photoperiodic conditions. While *Hd1* activates flowering by activating *Hd3a* in short days, it delays the flowering by repressing *Hd3a* in long days[71,72]. Homologues of the key floral meristem identity gene *LFY* have also been identified in grasses. Studies on the maize *ZFL* (maize *LFY* homologue), the rice *RFL* (rice *LFY* homologue) gene, and the rye grass *LtLFY* gene exemplify how homologues for a critically important *Arabidopsis* floral meristem identity gene have acquired distinct temporal and spatial domains of expression in the branched inflorescence meristems typical of grasses. This thereby can contribute to new functions in regulating inflorescence branching perhaps in addition to their evolutionarily conserved role in establishing a floral meristem[73–76]. In addition to varied expression profiles for *LFY* homologues in diverse species, recent studies of the protein, from many plant species, elucidate how changes in the conserved DNA-binding domain, over evolutionary time, could contribute to its likely diverse functions[77].

Functions for grass homologues for many of the other floral meristem identity genes discussed here still remain unknown. Further characterization of homologues for positive and negative meristem regulators from lower eudicot and primitive land plant species would shed light on the molecular evolution of plant body plan. Mounting evidence implicates the role of signalling molecules such as hormones and light in floral initiation besides their role in root and shoot development. Recent studies show an elegant correlation among auxin efflux, auxin gradient and plant primordia development[78]. One of the challenges ahead will be to mechanistically couple the mode of auxin action during root, shoot and flower development with the many different key regulators known to be involved in plant organ formation.

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