Review

Gene networks controlling the initiation of flower development

Frank Wellmer[1] and José L. Riechmann[2,3]

[1] Smurfit Institute of Genetics, Trinity College Dublin, Dublin 2, Ireland
[2] Center for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB, Barcelona 08034, Spain
[3] Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, 08010, Spain

The onset of flower formation is a key regulatory event during the life cycle of angiosperm plants, which marks the beginning of the reproductive phase of development. It has been shown that floral initiation is under tight genetic control, and deciphering the underlying molecular mechanisms has been a main area of interest in plant biology for the past two decades. Here, we provide an overview of the developmental and genetic processes that occur during floral initiation. We further review recent studies that have led to the genome-wide identification of target genes of key floral regulators and discuss how they have contributed to an in-depth understanding of the gene regulatory networks controlling early flower development. We focus especially on a master regulator of floral initiation in *Arabidopsis thaliana* APETALA1 (AP1), but also outline what is known about the AP1 network in other plant species and the evolutionary implications.

Flowering time control in *Arabidopsis*: integration by the network

In the life cycle of an angiosperm plant, the transition from vegetative to reproductive growth is a key developmental step that is under tight genetic control. To maximize reproductive success, the timing of this switch is coordinated with both the environment and the physiological state of the plant. Studies on the biology of flowering time (Glossary) in the model plant *Arabidopsis thaliana* have shown that the responses to various external and internal conditions are integrated by a complex gene regulatory network that controls this transition. Consequently, the regulation of flowering time has been a major adaptive trait during plant evolution and domestication. A large number of genes have been characterized as flowering time regulators, and several recent reviews have provided detailed descriptions of flowering time pathways [1–5] (Figure 1). Ultimately, all of these pathways converge on so-called floral meristem identity genes, which act by directing the meristems (or primordia) that form on the flanks of the inflorescence meristem to develop into flowers. Floral meristem identity genes code for transcription factors that are conserved across plant species [6] and include *Arabidopsis APETALA1* (*AP1*) (and paralogs) and *LEAFY* (*LFY*) [7,8]. Here, we provide a brief overview of flowering time control in *Arabidopsis* as an introduction to the gene networks that control the initiation of flower development. In particular, we will describe the central role that AP1 plays in the transition from floral induction to flower formation by acting as a switch between these two developmental programs and constituting a hub in the corresponding network of regulatory genes [9].

In *Arabidopsis*, flowering in response to seasonal changes is controlled by the vernalization, photoperiod and ambient temperature pathways, which act coordinately with those that respond to endogenous and developmental cues: the autonomous, gibberellin and age-dependent pathways (Figure 1). Long days accelerate flowering via the photoperiodic (day length) pathway, which is mediated by *CONSTANS* (*CO*). *CO* codes for a zinc finger and CCT-domain-containing transcription factor that accumulates under long day conditions in leaves as a result of the combination of the rhythmic expression of *CO* mRNA and the stabilization of CO protein by light [2]. CO activates the expression of *FLOWERING LOCUS T* (*FT*) [10],

Glossary

Autonomous pathway: flowering time pathway that controls the transition from the vegetative to the reproductive phases of development depending on the endogenous signals and independently of environmental cues.
Floral meristem: group of undifferentiated cells that are formed by the inflorescence meristems and that develop into flowers. In contrast to shoot meristems, floral meristems show determinate growth.
Floral meristem identity gene: regulatory gene that controls the specification of floral meristems during the onset of flower development. Examples of floral meristem identity genes are *LEAFY* and *APETALA1*.
Floral pathway integrator: regulatory gene whose expression is controlled by the activity of several flowering time pathways and that regulates the expression of meristem identity genes.
Florigen: mobile flowering signal that is produced in leaves and acts at the shoot apex. The protein FLOWERING LOCUS T has recently been identified as a key component of florigen.
Flowering time: time period between seed germination and the onset of flower development. Flowering time is tightly controlled to ensure that plants flower when conditions for reproduction are favorable.
Gibberellin pathway: flowering time pathway that controls the transition from the vegetative to the reproductive phases of development depending on the endogenous concentration of the phytohormone gibberellin.
Inflorescence meristem: shoot apical meristem after the transition from the vegetative to the reproductive phase of development. It produces floral meristems that develop into flowers.
MADS domain: conserved sequence motif found in a family of transcription factors. MADS domain-containing proteins form dimers as well as higher order protein complexes. Many floral regulatory genes encode MADS domain proteins.
Photoperiod: duration of light and dark periods during a day. In the laboratory, the photoperiods typically used are 8 h light, 16 h darkness ('short day') or 16 h light, 8 h darkness ('long day').
Vernalization pathway: flowering time pathway that promotes flowering in response to the exposure to prolonged periods of cold temperature.

Corresponding authors: Wellmer, F. (wellmerf@tcd.ie); Riechmann, J.L. (jlrgmp@cid.csic.es)

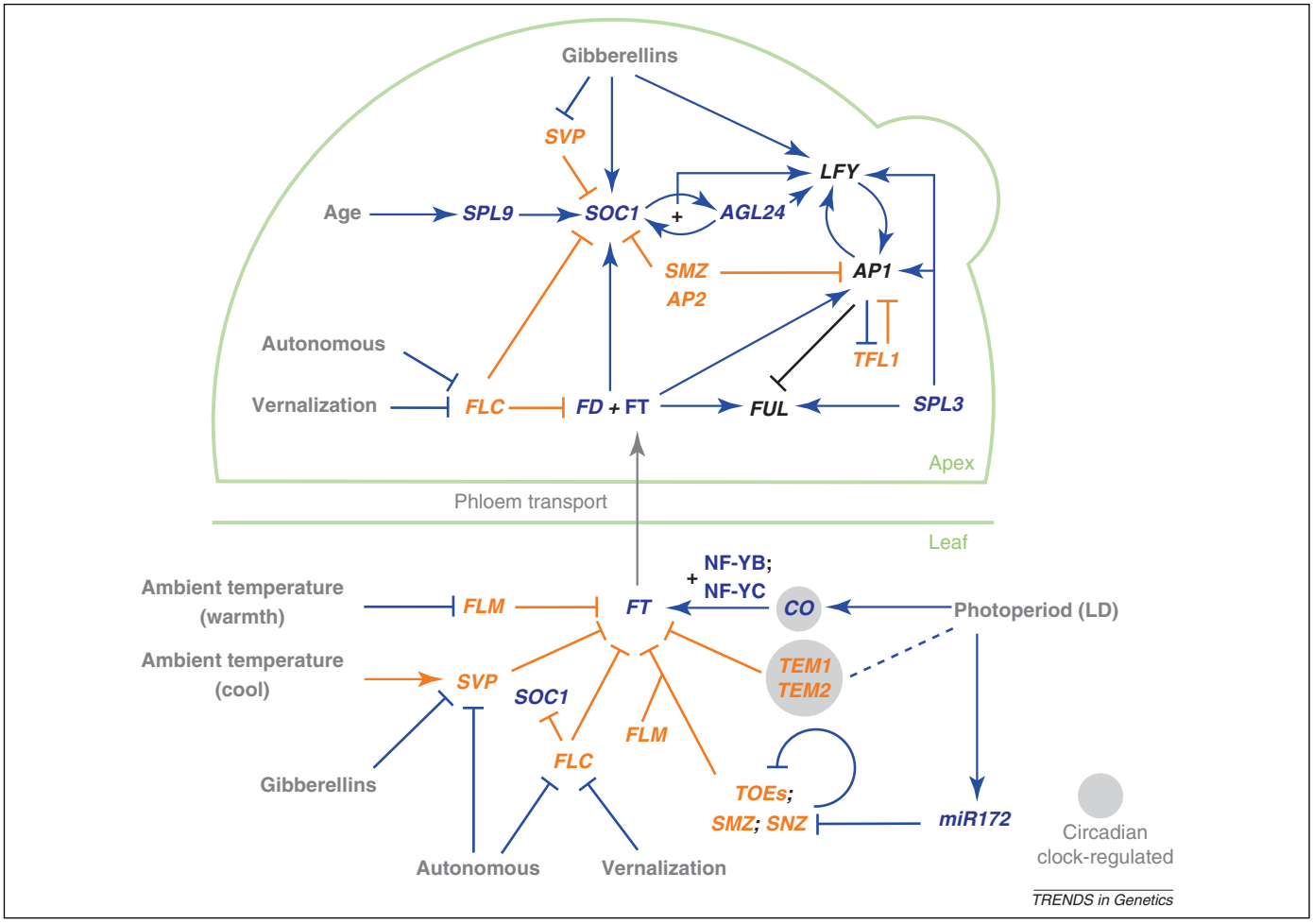

Figure 1. Schematic diagram of the genetic pathways that regulate flowering time in *Arabidopsis*. Different pathways respond to various external (photoperiod, vernalization, ambient temperature) and internal (autonomous, age, gibberellins) conditions to regulate the floral transition through an elaborate genetic network. Inductive signals have first to overcome the activity of several repressors of the floral transition [5] (genes indicated in orange), for activators (genes indicated in blue) to eventually turn on the meristem identity genes (*AP1* and *LFY*). The pathways operate on different network components (genes) and on different tissues, but the genetic network integrates their inputs through changes in expression of many of its genes. Inductive photoperiods (long days; LD) are perceived in the leaves and eventually result in the upregulation of *FT* by *CO* (a zinc finger and CCT domain-containing transcription factor), together with transcription factors of the NF-YB and NF-YC classes [14] (*FT* is also upregulated by warm temperatures independently of CO [32]). The expression of *FT* in the leaf vasculature [10] is a main determinant of the timing of flowering; therefore, its transcription is tightly regulated by many transcription factors as well as by the chromatin state at the *FT* locus [11,14]. Repressors of *FT* expression prevent precocious flowering and include: *FLC*, which integrates the autonomous and vernalization pathways [1,4]; the RAV transcription factors TEM1 and TEM2, which provide a quantitative balance to the activation activity of CO [15]; the AP2-domain proteins TOE1, TOE2, TOE3, SMZ and SNZ [16–18]; and the MADS domain factor SVP [29,31]. *FT* repression by *SMZ* in the leaves has been shown to depend on *FLM* [18], a MADS-box gene related to *FLC* that also mediates the induction of flowering by warm temperatures [32,33]. The FT protein is transported through the vasculature to the apex [14], where it acts as a potent floral inducer and triggers the floral transition network. There, FT interacts with the bZIP transcription factor FD and coordinately they upregulate the MADS-box genes *SOC1*, *AP1* and *FUL* [25,26]. Some of the same factors that regulate *FT* expression in leaves, such as FLC, SMZ and SVP, also form part of the transition network at the shoot meristem, where they participate in the regulation of the floral promoter and integrator *SOC1* (which, like *FT*, is under the control of multiple pathways [24]). AP2 also participates in *SOC1* repression [30]. At the shoot apex, and in addition to repressing *SOC1*, FLC represses *FD* [22]. Therefore, the vernalization-dependent downregulation of *FLC* enables *Arabidopsis* to flower by producing a systemic signal in the leaf (FT) and by conferring competence to the meristem to respond to it (by relieving the FLC-mediated downregulation of the FT partner *FD*) [22]. SOC1 forms a positive feedback loop with AGL24 [35], and the two factors might form a complex for the upregulation of *LFY* [36]. The floral meristem identity genes (*AP1* and *LFY*) are also upregulated by members of the SPL family of transcription factors [27,49]. Once activated or upregulated, the meristem identity genes control the initiation of flower development, with AP1 then repressing a substantial part of the floral transition network that regulates its own activation, including *SNZ*, *TOE1*, *TOE3*, *TFL1*, *AGL24*, *SVP*, *SOC1*, *SPL9*, *TEM1* and *TEM2*, and *FD* [9] (this repression is not represented in the diagram, see main text). Genetic interactions that promote flowering and genes of floral promoters are indicated in blue, and interactions that repress flowering and genes of floral repressors are indicated in orange (the meristem identity genes *AP1* and *LFY* are indicated in black); arrows represent promotion or gene activation and blunted lines represent gene repression. The dashed line connecting ''photoperiod'' and *TEM1/TEM2* indicates that the *TEM* genes form part of the photoperiod genetic pathway by providing a counterbalance to the activity of CO (their expression is regulated by the circadian clock and decreases over time). The main network components and interactions are depicted in the diagram, but additional elements have been omitted for simplicity.

probably by binding to *FT* regulatory regions and by interacting with transcription factors of the NF-YB and NF-YC classes [11–13]. The FT protein is a component of the mobile flowering signal 'florigen' that moves upon its expression in the vascular tissue of leaves to the shoot apex [14]. The capability of CO to induce *FT* expression is counteracted by several regulators that repress *FT* through different mechanisms or pathways, thereby preventing precocious flowering [5]. These so-called floral repressors include the RAV transcription factors TEMPRANILLO1 and 2 (TEM1 and TEM2) [15], the APETALA2 (AP2) domain proteins TARGET OF EAT 1–3 (TOE1–3), SCHLAFMÜTZE (SMZ) and SCHNARCHZAPFEN (SNZ) [16–19] and the MADS domain factor FLOWERING LOCUS C (FLC). *FLC* is a potent floral repressor that acts both in leaves and the apical meristem and, with its expression controlled by epigenetic mechanisms as well as at a direct transcriptional level, acts as the central

component of the vernalization response pathway [1,4]. Vernalization decreases *FLC* expression, which allows the induction of *FT* in leaves and the transition from vegetative to reproductive growth at the shoot apex, through the systemic signaling system that FT represents [20–22]. In addition, FLC represses two other floral promoters at the shoot apex, the MADS-box gene *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FD* [20–24]. *FD* codes for a bZIP transcription factor that physically interacts with FT, and coordinately they upregulate *SOC1* (the expression of which is an early marker for floral transition [24–26]). Thus, the vernalization-dependent downregulation of *FLC* enables *Arabidopsis* to flower by producing a systemic signal in the leaf (FT) and by conferring competence to the meristem to respond to it [22]. *SOC1* is also positively regulated by the gibberellin pathway, as well as by the age-related pathway, in this latter case via transcription factors of the SPL (SQUAMOSA PROMOTER BINDING FACTOR-LIKE) family, such as SPL9 [24,27,28]. The MADS-box gene *SHORT VEGETATIVE PHASE* (*SVP*) is another negative regulator of the floral transition, and *FLC* and *SVP* functions are in part mutually dependent: SVP interacts with FLC for the repression of *SOC1* in the apex and they might also together directly affect the expression of *FT* in the leaf [29] (the *flc svp* double mutant shows a stronger phenotype than either of the single mutants, indicating that these factors can also act independently; however, the *flc svp* phenotype is more similar to that of *svp* than of *flc*, implying that *FLC* is more dependent on *SVP* than vice versa [29]). *SOC1* expression at the apex is also directly repressed by AP2, as demonstrated recently through the genome-wide identification of AP2 targets (*AP2* is involved in several developmental processes, including floral organ determination and development, but has also been shown to act as a repressor of flowering) [30].

The expression of *SVP* is regulated by the autonomous and gibberellin pathways [29], as well as by ambient temperature, thereby mediating delayed flowering in cool conditions [31]. Conversely, elevated temperatures accelerate flowering, in a manner that is dependent on *FT* but not mediated by the main known *FT* activator CO [32]. Instead, the thermal induction of flowering largely depends on the MADS-box gene *FLOWERING LOCUS M* (*FLM*), a close paralog of *FLC* [32,33]. FLM is a floral repressor, and changes in its splicing patterns in warm temperatures might lead to a reduction in the levels of the FLM variant that represses *FT* [33].

In summary, the expression of both floral promoters (*FT* and *SOC1*) and floral repressors (*FLC* and *SVP*) is regulated by several flowering time pathways, thereby responding to multiple external or endogenous cues in each case. Another gene that participates in the integration of flowering signals in *Arabidopsis* is *AGAMOUS-LIKE24* (*AGL24*), the closest *SVP* homolog within the large MADS-box gene family [34], but which functions as a floral activator instead. AGL24 and SOC1 directly regulate each other's expression in a positive feedback loop [35], and the two factors might form a complex for the upregulation of *LFY* [36]. Thus, all these regulators of the floral transition form a small network with multiple interactions among themselves (and, undoubtedly, additional interactions still remain to be discovered), which is ultimately resolved in the upregulation of floral meristem identity genes (*AP1* and *LFY*; Figure 1). The term "floral pathway integrator" (FPI) was introduced to describe genes that upregulate the meristem identity genes, and whose expression is regulated by flowering pathways that sense environmental or developmental cues (i.e. changes in the expression of the FPI genes serve to integrate the different flowering inputs [4]). The term was initially applied to *FT*, *SOC1* and *LFY* (which is activated by gibberellins through both *SOC1*-dependent and independent mechanisms [24,37]), and later to *FD* and *AGL24*, for instance. However, the functional distinction between floral integrators and floral meristem identity genes is becoming increasingly blurred because flowering time genes such as *SVP*, *SOC1*, *AGL24* and *FT* have been found to participate in floral meristem identity determination and patterning [38–40]. Thus, integration emerges as a characteristic of the gene network rather than of the genes *per se*. In addition, recent studies have shown how the meristem identity genes, and *AP1* in particular, take part in the regulation of the floral transition network. A recent genome-wide identification of the transcriptional targets of AP1 has shown that it constitutes a hub in the regulatory network and that it acts as a developmental switch between floral induction and flower formation [9].

AP1 as an orchestrator of floral initiation

In *Arabidopsis*, floral primordia are initiated sequentially on the flanks of the inflorescence meristem. The outgrowth of these primordia and their positioning depends on the build-up of local concentration maxima of the phytohormone auxin [41]. Once floral meristem growth commences, a specific developmental program is activated that leads to the formation of the different types of floral organs in a stereotypic pattern. As outlined above, this key regulatory event is controlled by a small number of floral meristem identity genes, which were originally identified in mutant screens for plants with defects in early flower development. For example, plants in which *AP1* is disrupted exhibit a partial conversion of flowers into inflorescence-like structures [42]. The inactivation of an additional gene *CAULIFLOWER* (*CAL*) dramatically enhances the *ap1* mutant phenotype: the onset of flower formation is markedly delayed and inflorescence-like meristems accumulate at the shoot apices, leading to a cauliflower-like appearance [42]. This mutant phenotype indicates that *AP1* and *CAL*, which code for closely related MADS domain transcription factors [7,43], control the initiation of flower development in a redundant manner [42]. Flower formation is ultimately restored in *ap1 cal* double-mutant plants, probably by the misexpression of the gene *FRUITFULL* (*FUL*). *FUL* encodes a MADS domain protein that is closely related to AP1 and CAL and that is expressed in the shoot apical meristem at the transition to flowering [44]. *FUL* expression is negatively regulated by *AP1* in emerging floral meristems, and because of this negative regulation *FUL* is ectopically expressed in young floral primordia of *ap1 cal* double-mutant plants [44]. In the *ap1 cal ful* triple mutant,

Box 1. Function of LFY in floral meristem identity specification

LFY encodes a plant-specific transcription factor that acts as a main regulator of floral meristem identity [8]. In *lfy* mutants, flowers are partially replaced by shoot-like structures. When *AP1* activity is removed in addition to that of *LFY*, the conversion of flowers into shoots is almost complete, suggesting that AP1 and LFY have overlapping functions in the specification of floral meristems [8]. In agreement with this conjecture, the ectopic expression of both *LFY* and *AP1* leads to a replacement of shoots with individual flowers [91,92]. This result indicates that both genes can trigger the developmental program required for flower formation.

In contrast to *AP1*, whose expression commences in young floral meristems [7], *LFY* is already transcribed during vegetative growth in leaf primordia. However, its expression is strongly upregulated in incipient floral meristems by the activity of different flowering time pathways [93,94]. Therefore, *LFY* has been classified as a floral pathway integrator in addition to its role as a floral meristem identity gene [95].

Besides its function in regulating *AP1* and *CAL* expression (see main text), LFY is also known to be required for the activation of floral organ identity genes in specific domains of developing floral buds [96,97]. LFY function seems to depend on the availability of certain cofactors such as UNUSUAL FLORAL ORGANS (UFO) [98], WUSCHEL (WUS) [99] or SEP3 [39], but its exact mode of action is still poorly understood.

Genes acting downstream of LFY have been identified through the application of microarray analysis [19,47,100]. A small number of these genes have been further characterized as direct LFY targets, which has yielded preliminary insights into the LFY network. *LATE MERISTEM IDENTITY1* (*LIM1*) is a direct LFY target that encodes a homeodomain-Zip transcription factor and which functions as a meristem identity regulator and participates, together with LFY, in the upregulation of *CAL* upon floral transition [47,101]. However, for the vast majority of the identified genes, their possible functions in flower development are currently not known and, because of the lack of genome-wide binding data for LFY, it remains unclear in many cases whether they are direct LFY targets or constitute secondary response genes. The genome-wide identification of LFY target genes and the integration of the resulting datasets with those for AP1 should yield detailed insights into the gene regulatory networks underlying floral meristem identity specification.

flower formation is completely abolished [44] because the loss of *AP1* and *CAL* activities cannot be compensated by *FUL* misexpression. The regulatory interactions between these genes have complicated determining the functions of *FUL* during the development of wild-type inflorescences, but recent studies have shown that *FUL* mediates flowering time and meristem determinacy in coordination with *SOC1* [45].

Work to elucidate the molecular mechanisms through which these floral meristem identity genes specify floral primordia has so far mainly focused on *AP1*. The regulation of *AP1* transcription has been shown to be highly complex and to depend on the input of different flowering time pathways. *AP1* expression is initiated in incipient floral primordia [7] through at least three different mechanisms. One of them involves LFY [8], which also acts as a regulator of floral meristem identity (Box 1; for a recent review on *LFY*, see [46]). LFY has been shown to bind to the promoter of *AP1* (as well as that of *CAL* [47]) and to activate the expression of the corresponding gene [48]. A second mechanism for *AP1* activation depends on a protein complex formed by FD, which directly binds to the *AP1* promoter, and FT [25,26]. Recently, a third pathway that mediates *AP1* activation has been identified, which involves the direct binding of members of the SPL family of transcription factors to the regulatory region of *AP1* [27,49]. The *AP1*-promoting activities of these pathways are counteracted by several regulatory proteins that repress *AP1* expression. Examples are SMZ, which was found to directly inhibit *AP1* expression [18], and TERMINAL FLOWER1 (TFL1), a protein that is closely related to FT and that is thought to compete with it for binding to FD [50,51]. AP2, which is closely related to SMZ and also functions as a floral repressor, has also been shown to bind to *AP1* regulatory regions [30].

Despite the progress made in characterizing the pathways and mechanisms that regulate *AP1* expression during floral initiation, a detailed understanding of AP1 function has long been hampered by the fact that few of its target genes were known. The recent genome-wide identification of AP1 targets through a combination of microarray experiments and chromatin immunoprecipitation assays followed by next-generation sequencing (ChIP-Seq) has begun to fill this knowledge gap and led to the first detailed insights into the molecular mechanism of AP1 during floral initiation [9]. AP1 was found to bind to approximately 2000 sites in the *Arabidopsis* genome, but only a minority (~15%) of the genes in the immediate vicinity to those sites showed robust expression changes, either in response to AP1 activation or at more advanced stages of flower development (when additional AP1 cofactors might be present). Thus, many AP1-binding events seem to have no or only weak effects on gene expression. This limited overlap between DNA binding and transcriptional output might not be a peculiarity of AP1, but rather a relatively common feature for eukaryotic transcription factors (similar studies with other factors in *Arabidopsis*, as well as in animals, have also shown a low correlation, regardless of whether endogenous or mis-/overexpressed factors were targeted in the experiments [52]). This suggests that some, and perhaps even a majority, of the DNA-binding events by eukaryotic transcription factors might not be conductive to changes in gene expression.

Most of the identified AP1 target genes have been found to be downregulated, suggesting that AP1 acts predominantly as a transcriptional repressor during the initial phase of flower development. This result is in agreement with previous observations that indicated a preponderance of gene repression during floral induction [19,53]. It seems, therefore, that an important step for the onset of flower development is the downregulation of genes that are normally expressed in the inflorescence meristem. In particular, AP1 downregulates many of the known floral repressors including *TFL1*, *TOE1*, *TOE3*, *SNZ*, *TEM1*, *TEM2* and *SVP* (Figure 1). Thus, AP1 mediates floral initiation in part by overcoming the inhibitory effects of these genes. AP1 also downregulates additional genes that participate in floral transition, including some of its own activators: *SOC1*, *AGL24*, *SPL9*, *FD* and *FDP* (which is an *FD* paralog). In summary, a large fraction of the regulatory network that at the apex directs floral transition and the upregulation of *AP1* in the emerging primordia (Figure 1)

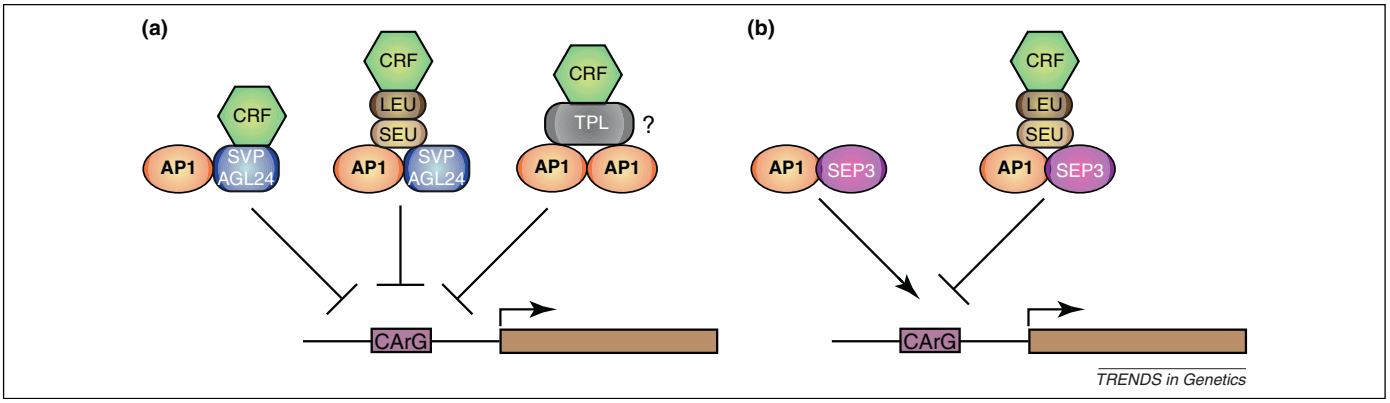

Figure 2. Molecular mechanisms underlying AP1 function during early flower development. **(a)** During the initiation of flower development, AP1 acts mainly as a transcriptional repressor [9]. Several different mechanisms seem to be involved in mediating this activity: firstly, AP1 can form heterodimers with the MADS domain proteins SVP and AGL24, which have been shown to interact with a transcriptional corepressor complex formed by the proteins SEU and LEU [54–56]. SEU/LEU can then recruit chromatin remodeling factors (CRFs) to target gene promoters [57]. AGL24 and SVP have also been shown to directly interact with chromatin remodeling factors [39], suggesting an additional mechanism through which AP1-containing transcription factor complexes might inhibit transcription. Lastly, AP1 is predicted to contain an EAR repressor domain in its C terminus [58], which might allow the recruitment of members of the TOPLESS (TPL) family of transcriptional corepressors [59]. A question mark indicates that this interaction has not yet been demonstrated. **(b)** After the onset of flower development, AP1 represses the expression of *SVP* and *AGL24* [9,53,61], which leads to the activation of the MADS-box gene *SEP3* through both direct and indirect mechanisms [9,39]. SEP3 is then thought to interact with AP1 and to activate the expression of genes involved in early floral organ formation [9]. However, it has also been shown that AP1/SEP3 heterodimers can interact with the SEU/LEU transcriptional corepressor complex [54–56], suggesting that AP1/SEP3 function can be reversed.

subsequently becomes under the control of, and is suppressed by, AP1. The fact that among the identified AP1 targets are a large number of genes that encode transcription factors (those mentioned above and many others that are still functionally uncharacterized) reflects the nature of AP1 as a regulatory hub.

AP1 was also found to control the expression of genes involved in a multitude of cellular and developmental processes, such as hormone responses and meristem patterning, but the exact roles that these genes play during early flower development are in most cases unknown. Elucidating the functions of the AP1 targets is likely to be a focus of future research and should yield new, detailed insights into the process of floral initiation.

From repression to activation: the role of AP1 interacting proteins

As outlined above, AP1 acts predominantly as a repressor during the initiation of flower development. Although the exact mode through which AP1 mediates the inhibition of target gene expression is currently unknown, available evidence suggests an involvement of several possibly independent mechanisms (Figure 2a). One of them is based on a transcriptional corepressor complex, which is composed of the proteins SEUSS (SEU) and LEUNIG (LEU), and that has been shown to interact with heterodimers between AP1 and one of the MADS domain proteins AGL24, SVP or SEPALLATA3 (SEP3) [54–56]. The SEU/LEU corepressor complex is thought to recruit proteins such as histone deacetylases to target genes [57], resulting in an altered chromatin structure and reduced promoter accessibility. Interestingly, SVP and AGL24 have also been shown to directly interact with proteins that affect histone methylation and acetylation, respectively [39], suggesting the existence of additional mechanisms by which AP1 target genes might be suppressed. Furthermore, AP1 contains a sequence in its C terminal region that is predicted to function as an ERF-associated amphiphilic repression (EAR) domain [58]. This domain might allow the recruitment of members of the TOPLESS protein family, which constitute another class of transcriptional corepressors [59].

As mentioned above, AP1 can interact with several MADS domain proteins including the flowering time regulators AGL24 and SVP as well as SEP3, which is a component of a large number of transcription factor complexes involved in flower development [60]. Notably, the expression of these AP1 partner proteins is under tight developmental control, suggesting that the formation of different AP1-containing complexes is essential for proper floral initiation. During the earliest stages of flower development, AP1 is thought to mainly interact with AGL24 and SVP [55], which are known to repress genes required for the specification of floral organs, including *SEP3* [39,55]. At the same time, the expression of both *AGL24* and *SVP* is gradually downregulated by AP1 [9,53,61], ultimately resulting in the activation of *SEP3* in early-stage floral buds [9,39,55]. SEP3 is then thought to interact with AP1 [38] (and possibly also with LFY [39]) and to activate genes required for the formation of floral organs, a process that commences shortly after the onset of *SEP3* expression. In agreement with the idea that AP1 and SEP3 act together in early flower development (Figure 2b), it was found that these two transcription factors have similar sets of target genes and preferentially bind to the same sites in the *Arabidopsis* genome [9]. Furthermore, joint target genes were found to be predominantly upregulated during more advanced stages of flower development when organ formation was initiated [9]. Therefore, it seems that AP1/SEP3-containing transcription factor complexes act by promoting the expression of genes that are required for early floral organ development. However, the known interaction between the AP1/SEP3 heterodimer and the SEU/LUG corepressor complex (see above) indicates that at least in some cases this activity can be altered.

Taken together, experimental evidence obtained to date suggests that AP1 acts mainly as a transcriptional repres-

sor during early flower development, but reverses its activity when SEP3 becomes available as a partner protein. This change in AP1's activity is thought to mediate the switch from floral induction to flower formation [9]. Understanding in molecular detail the exact mechanisms by which AP1 and its different interacting proteins function in a developmental context will be a main challenge of research for years to come.

Floral initiation in cereals: conservation and innovations of *AP1* function

Is the role of AP1 and that of other regulators involved in the control of floral initiation unique to *Arabidopsis* and its relatives, or is it conserved across flowering plants? Of particular interest in this context are the grass-like plants (monocots), which are distantly related to eudicots. Studies on cereals such as wheat and rice have demonstrated the existence of vernalization, photoperiod and autonomous flowering time pathways in monocots and identified several orthologs of *Arabidopsis* floral regulators in their genomes [3,4,62–64]. However, some of the genes that participate in the control of flowering time in cereals do not have close homologs in *Arabidopsis*, and orthologous genes might not play the exact same roles in monocots as in eudicots, indicating considerable differences in network circuitry.

The evolutionary history of the *AP1/FUL*-like MADS-box genes has been extensively studied. In *Arabidopsis*, the *AP1/FUL* clade comprises *AP1*, *CAL* and *FUL*: the *AP1* and *FUL* lineages originated from the duplication of an ancestral *AP1/FUL* gene that occurred at the base of the eudicots, whereas the additional duplication that resulted in *Arabidopsis AP1* and *CAL* was specific to the Brassicaceae [65]. By contrast, early in the evolution of monocots, a genome-wide duplication event resulted in two paralogous *AP1/FUL* genes (sometimes referred to as the *FUL1* and *FUL2* lineages) [65–67]. The complex history of gene duplications within the *AP1/FUL* clade during angiosperm evolution makes it difficult to establish clear orthologous relationships across plant families [67].

In wheat (and other temperate cereals such as barley), an *AP1/FUL* homolog has been shown to correspond to *VERNALIZATION1* (*VRN1*), which is involved in the control of flowering in response to vernalization (in conjunction with two other genes, *VRN2* and *VRN3*; Figure 3, Box 2) [68–70]. In winter wheat varieties, which require a long exposure to cold to flower in the spring, *VRN1* expression is induced by vernalization; by contrast, spring wheat varieties do not require vernalization and express *VRN1* in the absence of such treatment [68–71]. In addition to this role in the vernalization response, *VRN1* is required for floral initiation. A wheat strain carrying a deletion that encompasses the *VRN1* gene never produces flowers [72], and the reduction of *VRN1* expression by RNAi delays the apex transition to the reproductive stage [73]. The functional roles of *VRN1* correlate with its expression pattern. In winter cereals, *VRN1* is first expressed in leaves in response to vernalization and later in the apices [68,70,74]. In fact, *VRN1* (*FUL1*) and the paralogous gene *FUL2* are expressed in the inflorescence meristems of all studied grasses, indicating a conserved, general role in the transition to flowering [74]. Both genes are also expressed in spikelet and floret meristems, consistent with a role in floral meristem identity (spikelets are the short branches, specific to the grasses, that bear the florets, and the

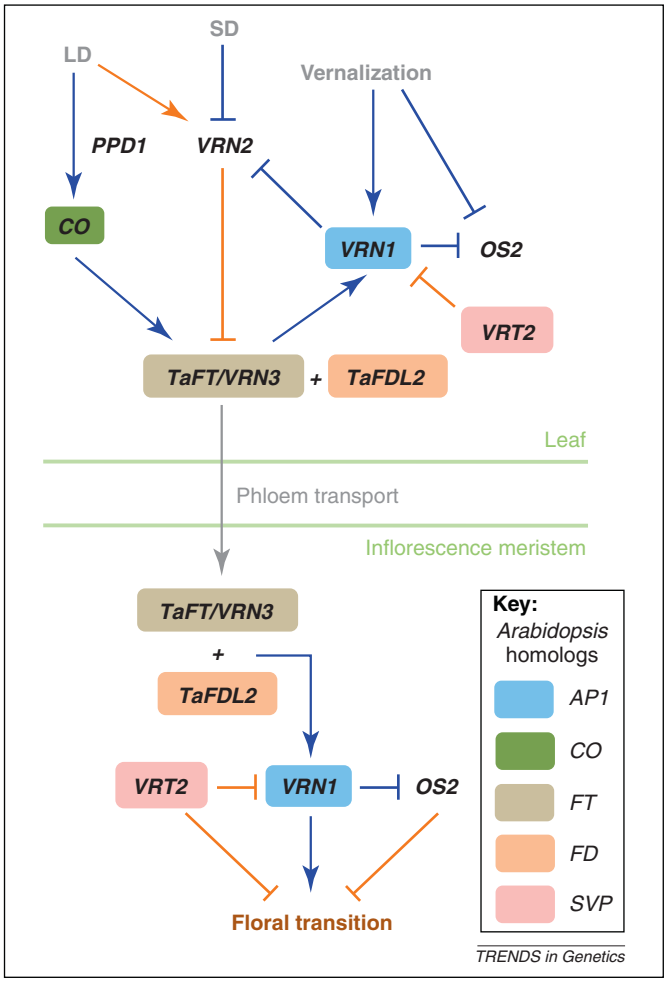

Figure 3. Schematic diagram of the genetic pathways that regulate flowering time in temperate cereals. The transition to flowering in winter varieties of temperate cereals (such as wheat and barley) requires vernalization and is promoted by long days (photoperiod) [64]. The floral repressor *VRN2* [81] and a repressed chromatin state of the floral activator *VRN1* [82] prevent floral induction. Vernalization relieves the chromatin-mediated repression of *VRN1* [82], and increased *VRN1* expression results in the repression of *VRN2* and activation of *VRN3/FT* [64] (*VRN1* is also upregulated during development [83]; not represented in the diagram). Therefore, *VRN1*, *VRN2* and *VRN3/FT* form a regulatory feedback loop that leads to the irreversible induction of flowering [64]. It has been shown that VRN3/FT interacts with FDL2, which binds to the *VRN1* promoter [84] (i.e. analogously to the FT–FD-mediated regulation of *AP1* in *Arabidopsis*). The activation of *VRN3/FT* requires long days and is mediated by *PPD1* (which codes for a pseudo-response regulator and controls the circadian timing of *CO* expression) and *CO* [85]. Day length is also a major determinant of the expression of *VRN2*, which operates in the photoperiod pathway to counteract floral induction and prevent flowering in the fall, when days are still long, in the absence of vernalization [64,83,86]. Conversely, the short days of winter result in the downregulation of *VRN2* (because the SD–LD induction of flowering is likely to be an ancestral trait, wheat and related grasses can be considered SD–LD plants) [64,86]. An alternative model for the regulatory feedback loop formed by *VRN1*, *VRN2* and *VRN3/FT*, where the interactions between these genes are reversed (with *VRN3/FT* repressing *VRN2*, *VRN2* repressing *VRN1* and *VRN1* activating VRN3/FT) has also been proposed [87]. However, subsequent detailed molecular characterization of the wheat *VRN1* deletion strain [72] in which this alternative model was based, strongly supports the model depicted here. The exact roles of the floral repressors *VRT2* (an *SVP* homolog [88,89]) and *OS2* (a MADS-box gene with weak similarity to *SOC1* [90]) in the floral transition network are still undefined (see main text). Genetic interactions that promote flowering are indicated in blue and those that repress flowering are indicated in orange; arrows represent gene activation (or the final promotion of the floral transition by *VRN1*) and blunted lines represent gene repression (or the repression of the floral transition by *VRT2* and *OS2*). LD, long days; SD, short days.

Box 2. Mechanisms of flowering time control in temperate cereals

The genetic and molecular characterization of flowering time genes in wheat has started to delineate the regulatory network of the floral transition in that species (Figure 3). *VRN2* codes for a protein with a C_2H_2-type zinc finger and a CCT domain that seems to be specific to grasses [81] and acts as a flowering repressor. *VRN3* is the wheat/barley ortholog of *FT* and, like *FT*, is upregulated in response to long days [85,102]. These two genes act with *VRN1* (see main text) in a regulatory loop that integrates day length (photoperiod) and vernalization inputs that promote flowering [72] (Figure 3). VRN3/*Ta*FT has been shown to regulate *VRN1* transcription through interactions with the bZIP protein FDL2 [84], analogously to the upregulation of *AP1* in *Arabidopsis* mediated by the FT–FD complex. *Ta*FT is a limiting factor in the activation of *VRN1*, whereas *FDL2* is expressed in leaves as well as in vegetative and reproductive apices [84] (in contrast to *Arabidopsis FD*, which is expressed in the shoot apical meristem [25]). Thus, the link that the FT–FD module provides between *AP1* expression and the flowering time pathways, with FT and FD accounting, respectively, for the temporal and for the spatial specificity of *AP1* expression upon the floral transition, is conserved in monocots and *Arabidopsis*. In addition, the *Ta*FT–FDL2 interaction provides a simple mechanism to account for the expression of *VRN1* in the leaves of temperate cereals (spring or vernalized winter varieties) [84]. Wheat and barley also contain homologs of *Arabidopsis SVP*, such as *TaVRT2*, which has been shown to be capable of repressing *VRN1* [88], and *BM1* and *BM10* [103], but their roles in the floral transition network are still under consideration (activity as a repressor participating in the vernalization response or as an inhibitor of floral meristem identity have both been considered) [64,88,103].

As outlined above, *Arabidopsis* AP1 directly downregulates genes that act as repressors of flowering [9] and, similarly, *VRN1* downregulates *VRN2* and *OS2*. *OS2* is a MADS-box gene that shows weak similarity to *Arabidopsis SOC1* (which is itself a floral promoter) and is expressed in leaves and shoot apices and repressed by vernalization and by *VRN1* [90]. In summary, although the molecular characterization of the *VRN1* regulatory network in temperate cereals is still limited, it seems that the nature of *AP1* as a network hub is maintained in *VRN1*.

corresponding floral meristems arise from the spikelet meristem) [74]. Thus, *VRN1* forms part of the floral transition network in a way that is both different (in leaves as a vernalization gene to induce flowering competency and in the shoot apical meristem to regulate the floral transition) and similar (as a reproductive meristem identity and floral organ development gene) to that of *AP1* in *Arabidopsis*. It has been proposed that the ancestral role for *AP1/FUL*-like genes in angiosperms was to confer floral meristem identity and to control floral organ development, and that their involvement in the transition to flowering is a derived character in the grasses [74]. In this respect, the vernalization pathway is supposed to have evolved independently in *Arabidopsis* and in the temperate cereals, given the differences between the two plants (no ortholog of *Arabidopsis FLC* has been identified in monocots [62]).

In contrast to temperate cereals, rice and maize do not rely on vernalization for flowering. Rice is a short day plant that blooms in late summer and in which the time of flowering critically depends on day length [75,76], whereas maize undergoes the transition to flowering after a fixed number of leaves has been produced (in contrast to its ancestor teosinte that, like rice, is induced to flowering by short days) [77]. The lack of vernalization response points to differences in the flowering time network between the rice and maize *AP1/FUL* homologs and wheat *VRN1*. However, the available data on maize and rice, albeit limited, indicate that the function of *AP1/FUL* homologs (*ZMM4/VRN1* in maize and *OsMADS14/FUL1*, *OsMADS15/FUL2* and *OsMADS18* in rice) in the shoot apical meristem to regulate floral transition, and as reproductive meristem and floral organ development genes, is conserved [76,78,79].

Altogether, the available data on flowering time and *AP1/FUL* genes in cereals highlight the existence of many aspects of the topology of the floral transition network that are conserved between dicots and monocots, as well as innovations, which can be common to either all monocots (the function of *AP1/FUL* genes in the shoot apical meristem to regulate the floral transition) or to a group of them (the role of *VRN1* in the vernalization response in temperate cereals). It can, therefore, be expected that the nature of AP1 as a network hub that was uncovered in *Arabidopsis* is also present in monocots.

Concluding remarks and future directions

Our understanding of the molecular mechanisms underlying floral initiation has begun to improve considerably in recent years aided by the development of technologies that allow the study of transcription factor function on a genome-wide scale. Although the identification of target genes of the floral pathway integrators and floral meristem identity factors discussed above is far from complete, it is clear from the limited number of examples currently available that this work will yield detailed insights into the compositions and architectures of the gene networks controlling floral initiation. One important step in understanding the behavior and dynamics of these networks will be the integration of the different large-scale datasets stemming from these experiments into comprehensive and predictive network models. To this end, pre-existing models can serve as a structural framework for network expansion [80]. Analysis of the flowering gene networks in plants different from *Arabidopsis* will shed light on the evolution of flowering pathways and, in the case of cereals, could provide invaluable information for generating new strains of crop plants with improved yields.

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