Leading Edge
Review

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The Timing of Developmental Transitions in Plants

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Plants rely heavily on environmental cues to control the timing of developmental transitions. We are beginning to better understand what determines the timing of two of these transitions, the switch from juvenile to adult vegetative development and the transition to flowering. In this review, we discuss how RNA silencing mechanisms may influence the juvenile-to-adult vegetative switch. We also describe the discovery and regulation of a component of "florigen," the mobile flowering promotion signal that is involved in the transition to flowering. Parallel themes are beginning to emerge from a molecular comparison of these two developmental transitions.

Introduction

Plants undergo several developmental transitions during their life cycle. The first is germination, which is the transition from the embryonic to the postembryonic mode of growth. After germination, the seedling passes through a juvenile vegetative phase, where it is not competent to flower. This is followed by the transition to the adult vegetative phase, where it can respond to floral inductive signals (Figure 1). With the transition to flowering, the plant enters the reproductive phase. Meiosis marks the transition to the gametophytic phase, and fusion of the gametes during fertilization starts the embryonic phase of the next generation. Many of these transitions are regulated by environmental cues to align development with favorable environmental conditions, thereby maximizing reproductive success. The last few years have seen significant advances in our understanding of how these developmental transitions are regulated, particularly the juvenile-to-adult vegetative transition and the transition to flowering. Here, we review these recent findings and discuss whether there could be mechanistic similarities in their regulation.

## Transition from Juvenile to Adult Vegetative Development

The environmental cues day length, light intensity, and ambient temperature, together with the plant hormone gibberellic acid, significantly influence the timing of the transition from juvenile to adult vegetative development, also referred to as the vegetative phase change (Figure 1) (Willmann and Poethig, 2005; Telfer et al., 1997). In some species, such as ivy and eucalyptus, there are prominent morphological and physiological differences between the juvenile and adult vegetative states whereas in others, such as *Arabidopsis thaliana* and maize, the phenotypic consequences are much more
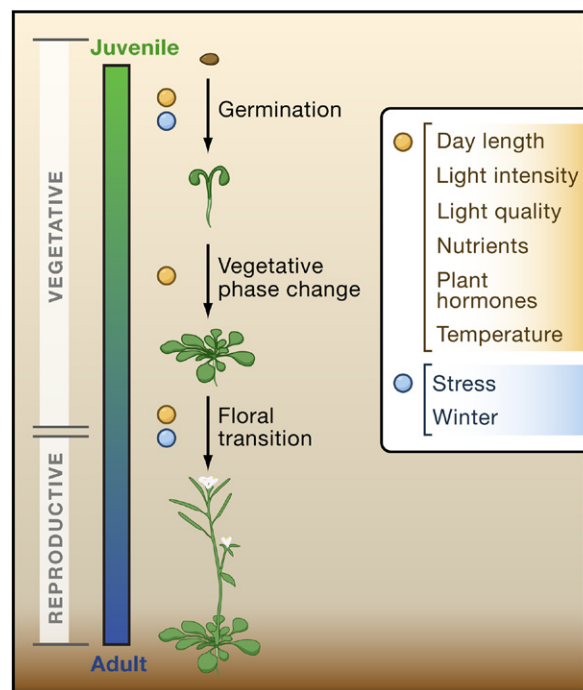
Figure 1. Developmental Transitions during the Plant Life Cycle

Germination is the transition between embryonic and postembryonic development. In the vegetative phase, the seedling progresses from the juvenile state into the adult state (vegetative phase change). The third major transition is the floral transition from the adult vegetative state to the reproductive state. Whereas the progression from the juvenile to the adult vegetative state is gradual, the floral transition is usually abrupt. All developmental transitions are regulated by environmental signals such as available nutrients, day length, light intensity, light quality, and ambient temperature as well as endogenous signals transmitted by plant hormones. Cold temperature and stress affect germination and the floral transition.

subtle. Recently, progress has been made in understanding this transition through its study in the genetically tractable systems maize and *Arabidopsis*. Juvenile maize plants have short leaves covered in epicuticular wax but no epidermal hairs, whereas the adult plant has long narrow leaves with hairs but no wax. Dominant gain-of-function mutations in the maize *Teopod* genes (*Tp1*, *Tp2*, and *Tp3*) prolong the juvenile phase but do not affect the onset of adult characteristics, suggesting that these genes function to promote juvenility (Dudley and Poethig, 1991; Poethig, 1988); however, these genes have not been cloned, and their precise function is unclear. Genetic mosaic experiments, where sectors of wild-type tissue were induced in *Tp1* or *Tp2* mutant plants, showed that Tp activity is likely to involve a non-cell-autonomous signal (Dudley and Poethig, 1993). The leaves formed during the vegetative phase change are composites of juvenile and adult tissue, with the base of the leaf displaying more adult traits and the tip more juvenile traits, suggesting that a signal is perceived directly in individual leaf primordia rather than in the shoot apical meristem (the plant growing tip). This hypothesis is strongly supported by clonal analysis of maize transition leaves and by the simultaneous rejuvenation of primordia in cultured maize shoots (Orkwiszewski and Poethig, 2000). This gradual change is also observed in *Arabidopsis*, where the vegetative phase change is quite subtle: Adult leaves are slightly more elongated, are curled downward with serrated margins and short leaf stalks, and have leaf hairs on the lower surface (Willmann and Poethig, 2005). Mutant analysis in *Arabidopsis* has implicated a role for gibberellins in the vegetative phase change, as mutants in gibberellin synthesis or response show a delayed transition (Telfer et al., 1997). However, the most informative mutants so far have been those showing a precocious onset of adult traits. Cloning of the corresponding genes has revealed
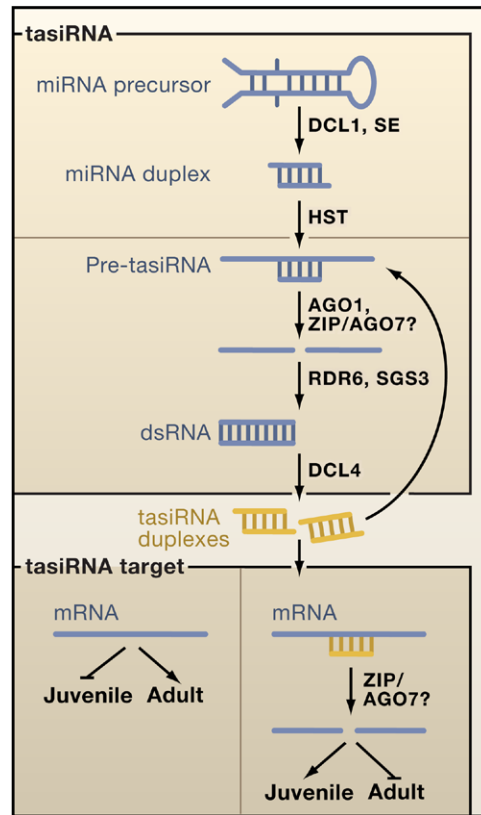
Figure 2. The Vegetative Phase Change Is Regulated by *trans*-Acting Small Interfering RNAs
A microRNA (miRNA) duplex is generated from a precursor involving DCL1 and SERRATE (SE) activity. HST is involved in exporting the miRNA into the cytoplasm, where it targets AGO1-dependent cleavage of the precursor of the *trans*-acting small interfering (tasi) RNA (pre-tasiRNA). One of the cleavage products is degraded, whereas the other is converted into dsRNA in an RDR6- and SGS3-dependent manner. *DCL4* then cleaves the dsRNA and generates tasiRNA starting from the end next to the site of miRNA-dependent cleavage. The miRNA duplex therefore sets the phase of the tasiRNA. The target gene product either promotes the adult phase or represses the juvenile phase. The tasiRNA induces degradation of the target mRNA, which results in either repression of the adult state or promotion of the juvenile state. Thus, when tasiRNA production is inhibited, the juvenile phase is shortened and the phase transition occurs prematurely. The identities of the tasiRNA and the tasiRNA target genes are not yet known.

an intriguing link between the vegetative phase change and RNA silencing pathways. These genes include *ZIPPY* (*ZIP/ARGONAUTE7*), which encodes an AGO-family protein (Hunter et al., 2003); *SERRATE*, a zinc-finger protein that has recently been shown to be required for the production of a microRNA (miRNA) (Clarke et al., 1999; Grigg et al., 2005; Prigge and Wagner, 2001); and *SQUINT* (*SQN*), which encodes the *Arabidopsis* homolog of cyclophilin 40, which is associated with the Hsp90 chaperone in many species (Berardini et al., 2001). Moreover, plants defective in *RNA-DEPENDENT RNA POLYMERASE6* (*RDR6*) and *SUPPRESSOR OF GENE SILENCING3* (*SGS3*), which were originally identified on the basis of their role in post-transcriptional gene silencing, also have an early onset of the adult phase (Peragine et al., 2004). A similar phase-change defect has been found in *dicer-like4* (*dcl4*) mutants (Gascioli et al., 2005; Xie et al., 2005; Yoshikawa et al., 2005).

The fact that loss-of-function *Arabidopsis* mutants reach the adult phase faster complements the interpretation from the dominant maize *Teopod* mutations that the function of the genes is to promote juvenility. Thus, current models are focused on the role of *trans*-acting small interfering RNA (tasiRNA) as part of a juvenility signal (Figure 2) (Allen et al., 2005; Vazquez et al., 2004; Yoshikawa et al., 2005). tasiR-NAs are endogenous small RNAs derived from a non-protein-coding gene that target degradation or cleavage of complementary mRNAs derived from another locus (Allen et al., 2005; Xie et al., 2005; Yoshikawa et al., 2005). The tasiRNA precursor itself is targeted by a miRNA and cleaved by AGO1. The cleavage product is then converted into dsRNA involving *RDR6* and *SGS3* and processed into tasiRNA by DCL4. Since DCL4 starts from the end that was generated by the miRNA-induced cleavage, all tasiRNAs have the same phase (see Figure 2). Although *ZIP* does not seem to

be directly involved in the production of the tasiRNAs studied so far (Yoshikawa et al., 2005), the vegetative phase-change defect seen in *zip* mutants and the genetic interaction with *RDR6* and *SGS3* suggest that *ZIP* also functions in this pathway. Apart from the phase-change defect and a weak flower defect, *rdr6*, *sgs3*, and *zip* mutants do not show any obvious developmental phenotypes (Hunter et al., 2003; Peragine et al., 2004). Mutations in the *HASTY* (*HST*) gene also accelerate the vegetative phase change. However, *hst* mutants display a number of other developmental defects (e.g., *hst* mutants delay the transition to flowering, but only in nonpromotive photoperiods; Bollman et al., 2003). *HST* encodes a homolog of the mammalian exportin5 nuclear export receptor and is thought to export miRNA into the cytoplasm (Park et al., 2005). Therefore, although *HST* clearly has a broad role in miRNA metabolism, during the vegetative phase change, it might specifically be required for the processing of the miRNA that sets the phase of the tasiRNA precursor, a hypothesis supported by genetic data showing that *hst* is epistatic over *sgs3*, *rdr6*, and *zip* (Hunter et al., 2003; Peragine et al., 2004).

Many interesting questions remain. What is the identity of the tasiRNAs, and are they the apparent signal predicted from the genetic mosaic experiments in maize? What are the genes targeted by the tasiRNAs, and do they promote the adult state or repress juvenility? Currently, we don't know whether the tasiRNAs repress accumulation of target mRNAs (as we expect) or perhaps promote their accumulation. Once the signal is known, it will become clearer whether temporal regulation accounts for the gradual change from juvenile to adult leaf production and whether the chimeric nature of leaves around the transition is brought about by a gradient in the competence of the cells to respond or by a gradient of the signal itself. How the environmental cues influence the timing of the phase change also remains to be investigated. As the generation of siRNA is inhibited by low temperatures (Szittya et al., 2003), it is tempting to speculate that the effect of ambient temperature on the vegetative phase change might be mediated through siRNA accumulation. Finally, it will be interesting to address whether the juvenility signal is related to the presumed small-RNA signal that defines the dorsoventral polarity of the developing primordia.

To date, the study of juvenile-to-adult vegetative transition has focused on the morphological phase change. Other aspects of juvenility include the competence to respond to floral inductive signals. Close relatives of *Arabidopsis*, including many of the *Brassica* species, e.g., brussels sprouts and cabbage, will not respond to vernalization (the acceleration of flowering induced by a prolonged period of cold temperature) until they have reached a certain developmental age. However, this phase appears to be very short in *Arabidopsis*, as imbibed seeds can be vernalized. Thus, the juvenile phase of the life cycle is likely to be composed of a number of perhaps overlapping stages that differ in length in different species.

The Transition to Flowering

The environmental inputs regulating the floral transition overlap considerably with those regulating the juvenile-to-adult vegetative transition (Figure 1). Physiological experiments provided the concept of multiple pathways that promote or repress flowering, all of which quantitatively contribute to an activity that switches the shoot apical meristem from producing leaves to forming flowers after reaching a threshold level (Bernier et al., 1993). Genetic dissection of flowering in *Arabidopsis* has supported this notion and identified the genes that constitute the multiple pathways. The floral pathways quantitatively regulate a common set of targets, the floral pathway integrators (*APETALA1*, *AP1*; *FLOWERING LOCUS T*, *FT*; *LEAFY*, *LFY*; and *SUPPRESSOR OF CONSTANS1*, *SOC1*), which in turn activate the genes needed for reproductive development (Simpson and Dean, 2002). This genetic framework can explain the diversity in flowering time in *Arabidopsis* accessions and even in many other species, although we have a long way to go before we fully understand the evolution of flowering control in all plants. The complexity of the framework has necessitated independent dissection of individual pathways, but the most recent data in the field, which we will concentrate on in this review, is exciting because it begins to integrate the individual promotion and repression pathways and reveals their site of action in the plant.

Production of the Photoperiodic Signal

Many plant species flower in response to changes in photoperiod, which serve to align flowering with favorable seasons—e.g., plants in northern latitudes flower in response to the increasing day lengths of spring, whereas plants like rice flower in response to short days to avoid drought periods. Photoperiod is perceived in the leaves, and a graft-transmissible signal is translocated to the shoot apex, where it stimulates the floral transition (reviewed in Zeevaart, 1976). In *Perilla*, production of the photoperiod signal is so stable that a single induced leaf can be grafted onto seven different plants successively and induce flowering in all of them (reviewed in Zeevaart, 1985).

The identity of this floral signal, named "florigen," has perplexed plant scientists for almost a century, but recent progress on identification of at least one component of it has come from analysis of the photoperiodic response in *Arabidopsis*. The *CONSTANS* (*CO*), *FT*, and *GIGANTEA* (*GI*) gene products promote flowering in response to long-day photoperiods (Koornneef et al., 1991). CO is a putative transcriptional regulator, and its expression is regulated at several levels
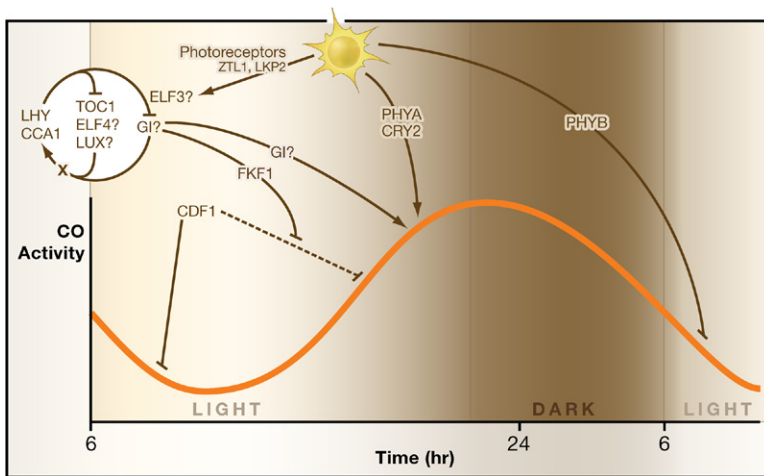
Figure 3. CO Protein Activity Is Regulated by the Circadian Clock and Light

The coincidence of light at the end of the (long) day and high CO protein levels activates the *FT* gene, which promotes flowering. The core oscillator of the circadian clock (upper left) constitutes a transcriptional/translational feedback loop and involves *LHY*, *CCA1*, *TOC1*, and possibly *ELF4*, *LUX*, and *GI*. During late evening, TOC1 and ELF4 activate expression of *CCA1* and *LHY*, so CCA1 and LHY levels peak at dawn. CCA1 and LHY repress the expression of *TOC1* (and possibly *ELF4*) through their binding to the "evening element" in their promoters. The decrease in TOC1 and ELF4 reduces *CCA1* and *LHY* expression during the day, which in turn releases the inhibition on *TOC1*. The cycle is closed by TOC1 and ELF4 accumulating in the evening, promoting the expression of *CCA1* and *LHY*. Mathematical modeling of the clock predicted two more components to account for the experimental data, and one of them could be *GI*. The clock is entrained by light through the classical photoreceptors and *ZTL* and *LKP2*, possibly via *ELF3*. The clock regulates *CO* transcription positively through *GI* and negatively through *CDF1*. *FKF1* releases *CDF1*-dependent repression of *CO* by degrading CDF1 specifically at dusk (hatched line). *PHYA* and *CRY2* stabilize CO protein in the afternoon, whereas *PHYB* destabilizes it in the morning, thereby further enhancing the circadian oscillation of CO activity.

(Figure 3) (Putterill et al., 1995; Samach et al., 2000; Suárez-López et al., 2001; Valverde et al., 2004). *CO* transcription is under circadian regulation through *GI* and *CYCLING DOF FACTOR1* (*CDF1*) activity (Imaizumi et al., 2005; Mizoguchi et al., 2005; Suárez-López et al., 2001). The plant circadian clock is composed of transcriptional/translational feedback loops involving *TIMING OF CAB EXPRESSION1* (*TOC1*), *EARLY FLOWERING4* (*ELF4*), *CIRCADIAN CLOCK-ASSOCIATED1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*), and *LUX ARRHYTHMO* (*LUX*) (Figure 3) (Alabadí et al., 2001; Doyle et al., 2002; Hazen et al., 2005; Schaffer et al., 1998; Strayer et al., 2000; Wang and Tobin, 1998). The clock is entrained by the well-characterized phytochrome and cryptochrome photoreceptors, as well as the ZEITLUPE (ZTL)/FLAVIN BINDING KELCH-REPEAT F-BOX1 (FKF1)/LOV KELCH PROTEIN2 (LKP2) class of proteins, which may function as photoperiodic and circadian photoreceptors (Figure 3) (Imaizumi et al., 2003; Yanovsky and Kay, 2003). The clock outputs are less well characterized but involve *FKF1*-dependent degradation of the CDF1 protein in the afternoon, thereby facilitating *CO* transcription, which was repressed by high CDF1 levels in the morning (Imaizumi et al., 2005). Light also regulates *CO* at the posttranscriptional level through *PHYTOCHROME* (*PHY*) *B* destabilizing CO protein in the morning and *PHY A* and *CRYPTOCHROME2* (*CRY2*) stabilizing it in the late afternoon (Valverde et al., 2004). Thus, only when clock-driven high levels of *CO* expression in the evening coincide with light-induced stabilization of CO protein under long-day conditions does the *CO* activity reach sufficiently high levels to activate flowering. These findings strongly support a model described as the external coincidence model for photoperiodism developed by Bünning (1936). It proposes that the circadian clock drives a rhythm in

a light-sensitive process and that in long-day plants, photoperiodic responses are promoted when the most sensitive phase of the endogenous rhythm overlaps with the light part of the day.

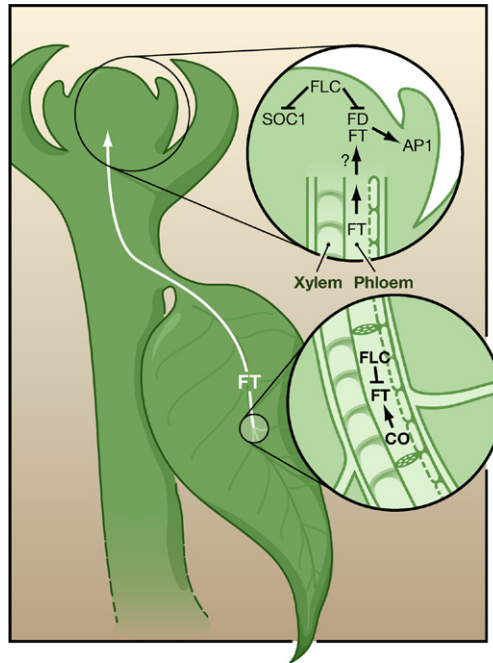## Identity of the Photoperiodic Signal

As perhaps anticipated from the grafting experiments described above, *CO* expression is required in the leaf vasculature to promote flowering (An et al., 2004). The targets of *CO* include *FT* and *SOC1* (Samach et al., 2000). Recent comparison of genes activated by shifting wild-type or *co* mutant plants from noninductive photoperiods into inductive photoperiods showed that only *FT* activation depends entirely on *CO* (Wigge et al., 2005). FT, a 20 kDa protein with some sequence identity to phosphatidylethanolamine binding protein or Raf kinase inhibitor protein (Kardailsky et al., 1999; Kobayashi et al., 1999), interacts with FD (a bZIP transcription factor), and the effect of *FT* overexpression is blocked in *fd* mutants (Abe et al., 2005; Wigge et al., 2005). *FT* is predominantly expressed in the vasculature, whereas *FD* is expressed at the shoot apex, suggesting that *FT* mRNA or protein may physically move in the plant from the leaf vasculature, where it is induced by *CO*, to the shoot apex, where it interacts with FD and stimulates the expression of *AP1*, which then activates flowering (Figures 4A and 4B). Expression of *FT* from a heat-shock-inducible promoter revealed that it is *FT* RNA that moves, as levels of *FT* RNA from the transgene increased at the shoot apex after heat shocking of a single leaf, whereas levels of a control RNA expressed from the same promoter did not (Huang et al., 2005). Thus, the current debate is whether it is *FT* RNA, protein, or both that move. Another question is whether the movement of proteins and RNAs in the phloem is specific to some proteins or RNAs or whether it is a general and potentially

unregulated phenomenon (Wu et al., 2002). Finally, how does *FT* RNA or protein get from the vasculature into the shoot apex where its interacting partner FD is expressed? Movement into the shoot apical meristem appears to be regulated, possibly by pathways involving RNA silencing (Foster et al., 2002; Schwach et al., 2005). In contrast, cell-to-cell trafficking of proteins within the shoot meristem seems to be the default state, and it may be that active subcellular compartmentalization is necessary to prevent it (Jackson et al., 1994; Sessions et al., 2000; Wu et al., 2003). Certainly in developing *Arabidopsis* embryos there is relatively free cell-to-cell movement (Stadler et al., 2005).

The experiments detailed above, in which transgenic *FT* expression was induced in the leaves, produced results similar to those from the physiological experiments first describing florigen. Firstly, the rate of translocation of *FT* could be analyzed; if the induced leaf was removed up to 3 hr after the heat shock, no flower-

**Figure 4. The Photoperiod and FLC Pathways Interact in the Floral Transition**
In favorable photoperiods, CO activates *FT* in the leaf veins, and this leads to the induction of flowering. *FT* RNA or FT protein moves in the phloem to the shoot apex, where its interaction partner FD is expressed. It is unclear how FT moves from the end of the vasculature into the meristem. At the shoot apex, FT and FD together activate *AP1*. FLC represses the floral transition by antagonizing *FT* upregulation in the leaf veins and *FD* and *SOC1* expression at the shoot apex. *AP1* and *SOC1* induce flowering.

of the shoot apical meristem. *FLOWERING LOCUS C* (*FLC*) had been identified as a central repressor of flowering in *Arabidopsis* (Michaels and Amasino, 1999; Sheldon et al., 1999) and is expressed in the shoot meristem and vasculature (Bastow et al., 2004; Sung and Amasino, 2004). Expressing *FLC* from either a phloem- or a shoot apical meristem-specific promoter has now established that *FLC* expression is required in both tissues for the full repression of flowering (Searle et al., 2006). In the phloem, *FLC* expression strongly reduced both *FT* and *SOC1* expression, and this was overcome by producing *FT* from a heterologous promoter, indicating that *FLC* represses *FT* transcription. In the shoot meristem, *FLC* was found to inhibit *FD* and *SOC1* expression, thereby affecting the competence of the shoot meristem to respond to the *FT* signal coming in from the leaves (Figures 4A and 4B). The regulation of flowering time is thus a balance between promotion from pathways such as the photoperiod pathway and repression con-

ing occurred, but after 5 hr, leaf excision had no effect (Huang et al., 2005). This rate appears similar to that determined in physiological experiments analyzing the photoperiodic flowering signal (Bernier et al., 1993). Secondly, in the heat-shock experiments, transcript levels of both the transgenic and the endogenous *FT* gene increased at the shoot apex (Huang et al., 2005). This can be interpreted as positive autoregulation of *FT* and offers an explanation for why the *Perilla* leaf from the induced plant could induce flowering on seven successive scions. Physiological experiments involving disruption of the vasculature below the induced leaf also predicted a signal that travels from the leaves to the roots, where it stimulates cytokinin production, which is then transported to the shoot apex (Bernier et al., 1993). Whether this signal involves *FT* remains to be tested. In summary, it appears that *FT* is one component of the physiologically defined florigen signal.

FLC Antagonizes Production and Action of Photoperiodic Signal
Recent progress has given insight into how the photoperiodic signal interacts with the floral repression pathway, which is considered to regulate the competence

ferred by *FLC*. *FLC* is likely to play additional roles in the floral transition, as the analysis of natural variation of the temperature compensation of the circadian clock found that *FLC* lengthened the period specifically at 27°C, possibly through the clock-associated transcription factor *LUX ARRYTHMO* (*LUX*) (Edwards et al., 2006; Hazen et al., 2005; Swarup et al., 1999).

Different Mechanisms Relieve *FLC* Repression
The major environmental pathway that represses *FLC* expression is vernalization. Vernalization occurs as plants experience weeks and months of low temperature (winter). In *Arabidopsis*, the optimum has been measured at 4°C (Napp-Zinn, 1969). *FLC* expression is reduced progressively during the cold and then remains stably low during subsequent growth in warm temperatures (Michaels and Amasino, 1999; Sheldon et al., 1999). Given that *FLC* strongly represses flowering, this slow reduction of *FLC* levels during winter aligns flowering with the favorable conditions of spring. A requirement for vernalization distinguishes winter annual and biennial plants (those that overwinter in a vegetative state) from summer annuals and rapid cyclers, the latter often managing several generations a year. All these differ-

Activators and Repressors of *FLC* Expression
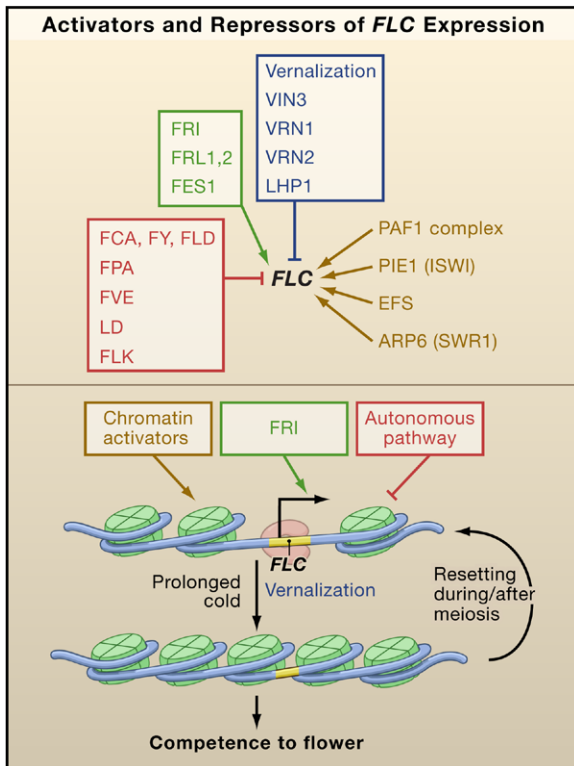
Figure 5. Regulation of *FLC* Expression

(Top panel) Expression of the floral repressor *FLC* is activated and re-pressed by many pathways. The autonomous pathway (red) represses *FLC* expression and comprises FCA, FY, FLD, FPA, FVE, LD, and FLK. Vernalization represses *FLC* through VIN3, VRN1, VRN2, and LHP1 (blue). FRI is a strong activator of *FLC* expression and acts together with FRL1, FRL2, and FES1 (green). Several chromatin regulators (brown) activate *FLC* expression (PAF1 complex, PIE1, EFS, and ARP6).

(Bottom panel) Regulation of *FLC* chromatin structure. *FLC* activa-tors promote an active chromatin state at *FLC*, whereas many com-ponents of the autonomous pathway promote an inactive chromatin state. Vernalization causes remodeling of *FLC* chromatin and accu-mulation of histone modifications characteristic of heterochromatin. During or after meiosis, high *FLC* expression is reset to restore the vernalization requirement in the next generation.

ent types can be found among the different *Arabidopsis* accessions, whose collection sites range from the Arc-tic Circle to the Cape Verde Islands near the Equator, demonstrating that different reproductive strategies are beneficial in different environments. The molecular evo-lution of the genes underpinning these adaptive changes is currently a very exciting area of research.

The stability of *FLC* repression through mitotic cell divisions after the plants have experienced the cold as well as the temporal separation of experiencing the cold and its effect (earlier floral transition) suggested an epigenetic basis for vernalization. The characteriza-tion of mutants impaired in the vernalization response has shown this to be the case (Figure 5). *VERNALIZA-TION2* (*VRN2*) is a homolog of the *Drosophila* Poly-comb group gene *Su(Z)12* (Birve et al., 2001; Gendall et al., 2001). Polycomb group (PcG) complexes in

Drosophila are involved in the epigenetic fixation of gene expression states that are established during early embryogenesis and then kept constant during development through modifying the chromatin at the target locus (Pirrotta, 1995). The Su(Z)12-containing PcG complex PRC2 is thought to be responsible for histone H3 lysine 27 and possibly H3 lysine 9 meth-ylation (Czermin et al., 2002; Müller et al., 2002), and *FLC* chromatin was found to accumulate these histone marks after vernalization (Bastow et al., 2004; Sung and Amasino, 2004). In animals, the methylated H3 lysine 9 binds the heterochromatin protein 1 (HP1), which is thought to stabilize the repressive methylation mark and recruit further complexes involved in the for-mation of heterochromatin (Bannister et al., 2001). The one *Arabidopsis* homolog of HP1, *LIKE HP1* (*LHP1*), is involved in vernalization-dependent repression of *FLC* (Mylne et al., 2006). Other components of the vernalization response are *VERNALIZATION1* (*VRN1*), which encodes a plant-specific protein with DNA binding domains that associates generally and stably through mitosis with *Arabidopsis* chromosomes (Levy et al., 2002; Mylne et al., 2006), and *VERNALIZATION INDEPENDENT3* (*VIN3*), a plant homeodomain pro-tein required for histone deacetylation at *FLC* (Sung and Amasino, 2004). *VIN3* is only expressed after an extended period of cold, suggesting that it is an impor-tant early component of the vernalization response, but it is not sufficient to initiate the process (Sung and Amasino, 2004). What distinguishes the early steps of vernalization from cold acclimation—the adaptation to low temperatures, which happens within minutes to hours of a temperature drop—is not yet known.

A second *FLC* repression pathway was identified through analysis of late-flowering mutants that still respond to photoperiodic cues and are sensitive to vernalization. They were called autonomous pathway mutants, and, to date, seven autonomous pathway genes have been cloned (Figure 5). They are either chromatin-associated proteins or (potential) RNA bind-ing/processing factors. *FVE* encodes an MSI1 (multi-copy suppressor of *ira* 1) homolog (Ausin et al., 2004; Kim et al., 2004) and *FLOWERING LOCUS D* (*FLD*) an amine oxidase homolog (He et al., 2003). Both *FVE* and *FLD* have been implicated in histone deacetylation complexes (Ausin et al., 2004; He et al., 2003). FCA, FPA, and FLOWERING LATE WITH KH MOTIFS (FLK) all contain putative RNA binding domains; the other-wise unrelated FCA and FPA proteins contain RNA rec-ognition motif (RRM) domains (Macknight et al., 1997; Schomburg et al., 2001), whereas FLK contains three K homology (KH) domains (Lim et al., 2004; Mockler et al., 2004). FY is homologous to Pfs2p, an essential poly-adenylation and 3′-end-processing factor from yeast (Simpson et al., 2003). Finally, *LUMINIDEPENDENS* (*LD*) encodes a homeodomain protein with unknown function (Lee et al., 1994). The autonomous pathway genes are likely to form a series of semiredundant

subpathways all targeting *FLC* (Koornneef et al., 1998; Michaels and Amasino, 2001; Sheldon et al., 2000). Genetic and biochemical analysis indicates that *FCA* and *FY* form one subpathway. FCA physically interacts with FY through its C-terminal WW domain, which is a well-known protein-protein interaction module (Simpson et al., 2003). Abscisic acid has been reported to bind to FCA and block this interaction (Razem et al., 2006). *FCA* negatively regulates its own expression in an *FY*-dependent manner by promoting the use of an internal polyadenylation site in the *FCA* transcript (Macknight et al., 2002; Quesada et al., 2003), and, under very low light, abscisic acid blocks this autoregulation (R.D. Hill, personal communication) (Razem et al., 2006). *fy* knockouts are embryonic lethal, whereas alleles lacking the FCA interaction domain only display the late-flowering phenotype (Henderson et al., 2005), suggesting that FCA might recruit selected transcripts to the canonical polyadenylation machinery.

FLC Upregulation to Repress Flowering

High levels of *FLC* expression make the plant incompetent to respond to factors promoting the transition to flowering. They occur naturally in many winter-annual *Arabidopsis* accessions and are often caused by a dominant allele of the *FRIGIDA* (*FRI*) gene (Figure 5) (Johanson et al., 2000). Active *FRI* is dominant over the *FLC*-repressing activity of the autonomous pathway genes, although this epistasis can be reversed, for example through manipulation of *FCA* expression levels (Quesada et al., 2003). Cloning of *FRI*, a coiled-coil-domain protein, did not reveal a potential mechanism for how it upregulates *FLC* expression (Johanson et al., 2000). The search for proteins that act in the same pathway as *FRI*, through analysis of mutants that suppress *FRI*-mediated *FLC* activation, has identified a range of *FRI*-specific and nonspecific suppressors (Figure 5), with the latter also blocking high *FLC* expression caused by mutations in autonomous pathway genes. The *FRI*-specific suppressors comprise two *FRI* homologs and a novel zinc-finger protein, and they appear to act cooperatively to upregulate *FLC* rather than in a linear pathway (Michaels et al., 2004; Schmitz et al., 2005).

The nonspecific *FRI* suppressors are generally required for high levels of expression of *FLC* and act independently of *FRI*. They comprise an array of chromatin-modifying proteins as well as proteins homologous to the yeast PAF1 (polymerase II-associated factor 1) complex (He et al., 2004; Oh et al., 2004), which coordinates transcription elongation with chromatin modification and 3′-end formation of the transcript (Rosonina and Manley, 2005). Among the chromatin-modifying proteins required for high *FLC* expression are the putative histone methyltransferase EFS (also described as SDG8) (Kim et al., 2005; Zhao et al., 2005); an ATP-dependent chromatin-remodeling protein of the ISWI and SWI2/SNF2 family (Noh and Amasino, 2003); and the ARP6 protein

(also described as ESD1), whose yeast homolog has been identified as a component of the SWR1 chromatin-remodeling complex (Choi et al., 2005; Deal et al., 2005; Martin-Trillo et al., 2006).

So, why do mutations in these generic chromatin regulators display predominantly flowering-time defects? One possible explanation is that because *FLC* acts as a quantitative repressor and the level of its expression is tightly regulated, small changes in gene expression already have visible phenotypic consequences, whereas they do not for genes that act more qualitatively. Alternatively, variation in flowering time is under such strong selective pressure that gene duplication events may have led to floral-specific chromatin regulators or loss of mechanistic redundancy. Despite the complexity we know today, there are still likely to be many more components and even additional pathways regulating *FLC* to be discovered. Mutations in many of the components of the RNAi machinery (e.g., *DCL1*) lead to increased *FLC* expression, and, in most cases, their action has not yet been placed in a known pathway (F. Liu and C.D., unpublished data). *FLC* expression is therefore tightly regulated, and this in turn regulates the timing of the transition to flowering.

A Common Conceptual Framework Underlying the Juvenile-to-Adult and the Floral Transitions?

Our knowledge of the molecular basis of the juvenile-to-adult vegetative phase change and the transition to flowering is improving, and parallels between the two transitions have begun to emerge. Both are significantly modified by similar environmental conditions and gibberellins. Both involve transmissible signals originating from outside the shoot apical meristem. Both transitions are regulated by repressive pathways that prevent the transition from occurring precociously. Many genes, like *HST* and genes of the autonomous and photoperiod flowering pathways, function in both transitions, whereas *FT*, *SQN*, *TFL1*, and overexpression of *LEAFY* affect one but not the other. It will be interesting to see whether, as our understanding develops, both transitions are regulated through similar mechanisms, namely (1) production of a diffusible, potentially RNA, signal and (2) its movement to the apex and antagonistic action against repressors to activate a set of downstream genes that cause the transition to occur. Quantitative changes of a signal can provoke either a gradual response or an abrupt one, depending on whether the sensor responds continually or only after the signal has reached a certain threshold. Evolutionary modulation of these responses may explain the evolution of both transitions and the variability observed in different plant species.

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