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The quest for florigen: a review of recent progress

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Abstract

The photoperiodic induction of flowering is a systemic process requiring translocation of a floral stimulus from the leaves to the shoot apical meristem. In response to this stimulus, the apical meristem stops producing leaves to initiate floral development; this switch in morphogenesis involves a change in the identity of the primordia initiated and in phyllotaxis. The physiological study of the floral transition has led to the identification of several putative floral signals such as sucrose, cytokinins, gibberellins, and reduced N-compounds that are translocated in the phloem sap from leaves to the shoot apical meristem. On the other hand, the genetic approach developed more recently in *Arabidopsis thaliana* allowed the discovery of many genes that control flowering time. These genes function in 'cascades' within four promotive pathways, the 'photoperiodic', 'autonomous', 'vernalization', and 'gibberellin' pathways, which all converge on the 'integrator' genes *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (SOC1) and *FLOWERING LOCUS T* (*FT*). Recently, several studies have highlighted a role for a product of *FT* as a component of the floral stimulus or 'florigen'. These recent advances and the proposed mode of action of FT are discussed here.

Key words: *Arabidopsis*, flowering, *FLOWERING LOCUS T*, floral stimulus.

Introduction

Plants are sessile organisms, and therefore the location at which a seed germinates determines where the plant will grow and reproduce. To ensure reproductive success, flowering is controlled so that it occurs in the optimal environmental conditions for seed production. Classical physiological experiments have demonstrated that, in temperate species, environmental cues exhibiting seasonal variations such as photoperiod, temperature, and rainfall, and the developmental stage of the plant are important factors regulating the floral transition (Hastings and Follet, 2001). All of these environmental factors are not perceived by the same plant organs; for example, daylength is perceived by mature leaves and winter cold by the shoot apex (Bernier, 1988). Since flowering occurs in the shoot apical meristem (SAM), floral signals, also called the 'floral stimulus', are produced in response to daylength and translocated from the leaves to the SAM where they induce floral evocation that switches the SAM from leaf production to the initiation of flower buds (Knott, 1934; Zeevaart, 1976; Bernier and Périlleux, 2005; Corbesier and Coupland, 2005). In photoperiodic species, plants that are induced to flower by exposure to long days (LDs) or short days (SDs) can be induced with the appropriate daylength. This approach allowed the process to be subdivided into two successive steps: the 'induction' mechanisms that occur in the leaf and floral 'evocation' that consists of the events occurring in the SAM that commit it to form flowers (Evans, 1969).

Grafting experiments have clearly demonstrated that, in response to induction, floral signals are indeed produced in the leaves. For example, in *Perilla*, grafting of a single induced leaf onto an uninduced shoot was sufficient to induce flowering (Zeevaart, 1985). The pattern and velocity of movement of the floral stimulus also appeared to be very similar to that of assimilates, indicating that it is transported through the phloem (King *et al*., 1968; King and Zeevaart, 1973). Although transport of the floral stimulus across graft junctions could be followed indirectly by its effect on flowering, the identity of the stimulus was difficult to establish despite extensive studies that mainly revealed correlations more than direct identification of a causal agent (reviewed in Bernier and Périlleux, 2005; Corbesier and Coupland, 2005).

Molecular-genetic studies in the quantitative LD-plant *Arabidopsis thaliana* have made progress in identifying genetic pathways and regulatory proteins associated with

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the control of flowering time (Mouradov *et al*., 2002; Yanovsky and Kay, 2003; Boss *et al*., 2004; Jack, 2004; Putterill *et al*., 2004; Searle and Coupland, 2004). Specifically in the photoperiodic control of flowering, models have been proposed in which circadian clock control of gene transcription and post-transcriptional regulation of protein stability by light can combine to confer control of a regulating pathway that mediates the induction of flowering by day length (Searle and Coupland, 2004). Recently, several papers have been published aimed at the identification of the mobile signal acting downstream of leaf-induction and triggering flowering at the SAM. Based on molecular and genetic evidence, a product of the *FLOWERING LOCUS T* (*FT*) gene, particularly the mRNA, has been implicated as this signal and these advances are reviewed here.

Physiological approaches towards the identification of the floral stimulus

Early in the 20th century, Chailakhyan proposed that the floral stimulus is a universal, unique, and specific hormone called 'florigen' (Chailakhyan, 1937) but, despite extensive studies, such a compound was never isolated. Later, alternative theories were proposed and, using photoperiodic species that can be induced to flower by exposure to a single inductive photoperiod such as *Sinapis alba*, *Lolium temulentum*, *Pharbitis nil*, and *Xanthium strumarium*, physiological study of the floral transition led to the identification of several putative floral signals such as sucrose, cytokinins, gibberellins (GAs), and reduced N-compounds, that are translocated from leaves to the SAM in response to exposure to appropriate daylengths. Interestingly, these compounds induce in the SAM some of the cellular and molecular events typical of floral evocation (reviewed in Bernier and Périlleux, 2005). However, all these signals do not act, or are not all of equal importance in all species studied. For example, despite GAs being a primary factor in *Lolium* (King *et al*., 2001), they are not involved in *Sinapis alba* (Corbesier *et al*., 2004). This supported a theory known as the 'multifactorial control hypotheses' which proposed that several factors, promoters and inhibitors, belonging to the classes of nutrients and hormones, are involved in the control of the SAM floral transition and that genetic variation as well as past and present growing conditions result in different factor(s) becoming limiting in different genotypes or in a given genotype in various environments (Bernier, 1988).

Genetic control of flowering time in *Arabidopsis*

In addition to the physiological studies, the genetic approach developed more recently in *Arabidopsis*, a quantitative LD and facultative vernalization-requiring plant, allowed the discovery of many genes that control flowering time (reviewed in Boss *et al*., 2004; Searle and Coupland, 2004; Bernier and Périlleux, 2005; Corbesier and Coupland, 2005). To identify genes that control the floral transition, mutants that showed accelerated or delayed flowering under different conditions, commonly known as flowering-time mutants, have been isolated (Redei, 1962; Koornneef *et al*., 1991). These mutants were grouped according to their responses to various physiological conditions and then integrated into genetic pathways to explain the control of flowering time. Four main promotive pathways were identified in *Arabidopsis*: the 'photoperiodic', 'autonomous', 'vernalization', and 'GA' pathways. In addition to these four main pathways, less dramatic changes in ambient conditions also strongly influence flowering time. For example, exposure to lower temperatures (16 °C) delays flowering compared with the effect of growing plants at typical growth temperatures of 20–24 °C, and exposure to high ratios of far-red to red light associated with shading conditions accelerates flowering (Blázquez *et al*., 2003; Cerdan and Chory, 2003). Interestingly, all these pathways appear to interact in a complex manner and converge to regulate genes that are often referred to as 'floral integrators', *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) and *FT*, that act upstream of the genes involved in floral morphogenesis such as *APETALA1* (*AP1*) and *LEAFY (LFY*) (Moon *et al*., 2003; Pineiro *et al*., 2003; Takada and Goto, 2003) (Fig. 1). *FT* encodes a protein with similarity to the RAF kinase inhibitors of animals (Kardailsky *et al*., 1999; Kobayashi *et al*., 1999) whereas *SOC1* encodes a MADS box transcription factor (Borner *et al*., 2000; Lee *et al*., 2000; Samach *et al*., 2000).

Specifically in the photoperiodic control of flowering, a molecular hierarchy has been defined. Two flowering-time genes specific to this pathway are *GIGANTEA* (*GI*) and *CONSTANS* (*CO*). The *GI* gene encodes a large protein that is present in the nucleus and is highly conserved in Angiosperms and Gymnosperms but has no animal homologues (Fowler *et al*., 1999; Park *et al*., 1999) while *CO* encodes a B-box zinc finger protein that promotes transcription of downstream flowering-time genes (Putterill *et al*., 1995; Robson *et al*., 2001). The biochemical function of GI is unknown, but *gi* mutations cause severe late flowering (Redei, 1962), while overexpression of *GI* causes early flowering (Mizoguchi *et al*., 2005). GI regulates flowering time at least in part by the regulation of *CO* mRNA abundance; *gi* mutants contain less *CO* mRNA (Suarez-Lopez *et al*., 2001) while *GI* overexpressors show higher *CO* mRNA abundance. The abundance of *GI* and *CO* mRNAs is circadian clock regulated. Under LDs of 16 h light, in which these genes promote early flowering, *GI* mRNA abundance peaks around 10–12 h after dawn, whereas *CO* mRNA abundance rises around 12 h after dawn and stays high throughout the night until the following dawn (Fowler *et al*., 1999; Park *et al*., 1999; Suarez-Lopez

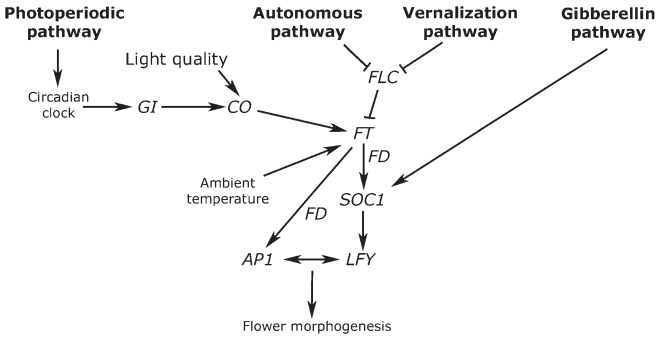

Fig. 1. Simple model of the four pathways controlling flowering time in *Arabidopsis thaliana*. The photoperiod pathway promotes flowering specifically under LDs. The transcription of the *GI* and *CO* genes is regulated by the circadian clock, whereas light quality regulates CO protein abundance. The autonomous pathway negatively regulates the abundance of the mRNA of the floral repressor FLC. *FLC* mRNA abundance is also repressed by vernalization independently of the autonomous pathway. Finally, gibberellin promotes flowering of *Arabidopsis*, particularly under SDs. All four pathways appear to converge on the transcriptional regulation of the floral integrator genes *FT* and *SOC1* which promote expression of *AP1* and *LFY*, genes required to confer floral identity on developing floral primordia. Figure adapted from Corbesier and Coupland (2005): Corbesier L, Coupland G. 2005. Photoperiodic flowering of *Arabidopsis*: integrating genetic and physiological approaches to characterization of the floral stimulus. *Plant, Cell and Environment* **28,** 54–66, and reproduced by kind permission of Blackwell Publishing.

et al., 2001). *CO* mRNA abundance is therefore high when plants are exposed to light at the end of a LD. *CO* expression is also regulated at the post-transcriptional level, so that the cryptochrome and phytochrome A photoreceptors act at the end of the day to stabilize the CO protein (Valverde *et al*., 2004), whereas in darkness the protein is rapidly degraded, probably as a consequence of being ubiquitinated. Under SDs the *CO* mRNA is only expressed in the dark, and so the protein would be predicted never to accumulate. In agreement with these data, in wild-type plants *FT* is activated by CO under LDs, but not under SDs (Suarez-Lopez *et al*., 2001; Yanovsky and Kay, 2002). Therefore, the combination of circadian clock-mediated regulation of *CO* mRNA abundance, and stabilization of CO protein by exposure to light can explain how CO promotes *FT* expression and, thus, flowering only under LDs.

The observation that CO is a major part of the molecular mechanism by which *Arabidopsis* discriminates between LDs and SDs suggests that CO is involved in the induction process and thus may act in the leaf to regulate the transition to flowering occurring at the apex. The *CO* mRNA is present at very low abundance, but is expressed widely (Putterill *et al*., 1995; Simon *et al*., 1996; Takada and Goto, 2003; An *et al*., 2004). Several recent observations have shown that CO acts in the vascular tissue and not the meristem to promote flowering. Specifically, triggering the expression of *CO* in the companion cells of the minor veins of the phloem of the mature leaves, using the promoter of a gene from melon encoding galactinol synthase, complemented the *co-1* mutation (Ayre and Turgeon, 2004). Independently, An *et al*. (2004), using the phloem companion cell specific promoter of the *Arabidopsis SUC2* sucrose-H^+ symporter gene (Truernit and Sauer, 1995), obtained similar results and, in addition, showed that expression of *CO* from meristem-specific promoters had no effect on flowering. Therefore, *CO* appears to act specifically in the vascular tissue to regulate the synthesis or transport of a long-distance signal that initiates floral development at the apex.

The mechanism by which CO acts to promote flowering in the phloem partially involves the *FT* gene. In wild-type plants, *FT* is expressed in the phloem, as detected using *FT::GUS* reporter constructs. Furthermore, *FT* expression is increased in the early flowering *terminal flower 2* (*tfl2*) mutant, and in particular is expressed at higher levels in the vascular tissue, suggesting that CO may activate its target gene directly in these tissues (Takada and Goto, 2003). In the phloem of *SUC2::CO* plants, *FT* mRNA abundance was increased in the phloem and *ft* mutations strongly suppressed the early flowering of *SUC2::CO* (An *et al*., 2004). Overexpression of *CO* in a *ft-10* mutant resulted in a late flowering phenotype, similar to that of the *co* mutant under long photoperiod, suggesting that inactivation of *FT* suppresses almost completely the signalling from *CO* and that *FT* is the major downstream target of *CO* (Yoo *et al*., 2005). Furthermore, expression of *FT* in the phloem from the *SUC2* promoter complemented the *co* mutation. However, in contrast to *CO*, *FT* promoted flowering when expressed in the meristem and the epidermal layer, as well as the phloem (An *et al*., 2004). Interestingly, among the 2000 genes activated or repressed in *Arabidopsis* leaves within an 8 h period after exposure to a single 16 h LD, only three genes responded differentially between WT and the *co* mutant and only one, *FT*, does not respond at all to the LD suggesting that *FT* is the major primary target of *CO* in leaves (Wigge *et al*., 2005). This is in agreement with the suppression of the early flowering phenotype of *CO*-overexpressors carrying an almost-null allele of *FT* whereas mutation of *SOC1* only partially suppressed early flowering (Yoo *et al*., 2005).

These data indicated that a major role of *CO* in flowering control is to activate *FT* in the leaf, and the observation that *FT* activates flowering when expressed in the leaf or the SAM suggested that a product of *FT* might be transferred to the SAM to activate flowering. However, these data are also consistent with FT activating synthesis of a floral promoting compound in the leaf or SAM (An *et al*., 2004).

Mode of action of FT

FT interacts with the bZIP transcription factor FD in yeast (Abe *et al*., 2005; Wigge *et al*., 2005). Mutations in *FD* cause late flowering and the *FD* mRNA is detected in the shoot apex and *FD* expression increased with age in both SD- and LD-grown plants. FD appears restricted to the

nucleus while FT is detected both in the nucleus and the cytoplasm (Abe *et al*., 2005). Mutation of *FD* strongly suppressed the early flowering phenotype of *35S::FT* suggesting that *FD* and *FT* might interact in plants. The fusion of the VP16 activation domain to FT supports this proposal: *35S::FT-VP16* induced extreme early flowering in a *ft tfl1* double mutant. These plants also had an increased expression level of *AP1* (Wigge *et al*., 2005). All these data support a model in which FT acts in the nucleus as part of a transcriptional complex with FD to activate the expression of the MADS-box transcription factor *AP1* in floral meristems (Abe *et al*., 2005; Wigge *et al*., 2005). In agreement with this hypothesis, the *AP1* mRNA level is reduced in the *fd lfy* double mutant, which also exhibits an inflorescence phenotype indistinguishable from the *ft lfy* double mutant suggesting that FD and FT are together involved in the up-regulation of *AP1* redundantly with *LFY* (Ruiz-Garcia *et al*., 1997; Abe *et al*., 2005). Independently, Wigge *et al*. (2005) reached the same conclusion and mapped a FD-response element in the *AP1* promoter at the same location as the LFY binding site. Interestingly, *AP1* expression is found in the vascular-rich region where *FT* is known to be expressed, is increased in *35S::FD* and this is *FT* dependent since it is abolished in a *ft* mutant.

However, *AP1* is unlikely to be the FT target in the SAM involved in flowering control because *ap1* mutants are not late flowering (Page *et al*., 1999). On the contrary, mutation in *SOC1* results in late flowering and the up-regulation of that gene in the SAM is one of the earliest events characteristic of the floral transition (Borner *et al*., 2000; Samach *et al*., 2000). Mutation in *FT* delays strongly the expression of *SOC1* in the SAM, even in plants overexpressing *CO*, and the direct expression of *SOC1* in the SAM is able to promote flowering even in the absence of *CO* or *FT* indicating that *SOC1* acts downstream of *FT* in the SAM (Searle *et al*., 2006). The use of plants overexpressing *FT* and carrying a *SOC1::GUS* reporter gene support this hypothesis (Yoo *et al*., 2005). A high GUS signal was observed in the apex and only a weak increase was seen in the vascular bundle of the cotyledons suggesting that *SOC1* is indeed downstream of *FT*, but the effect of *FT* on its activation seems restricted to the SAM. Finally, the activation of *SOC1* through *FT* appears to be *FD*-dependent since a mutation of either *FD* or *FT* reduced and delayed *SOC1* expression in the SAM (Searle *et al*., 2006).

A model that emerges from all these results can be summarized as follows. Photoperiodic induction occurs in the leaves and activates *CO* that stimulates *FT* expression. *FT* expression is not detected in the SAM but only in the vascular tissue suggesting that the *FT* mRNA or protein or both move to the SAM where FT interacts with FD to up-regulate *SOC1* within hours of floral induction. Later FD/FT act redundantly with *LFY* to activate *AP1*. Another possibility concerning the first events occurring in the leaves in response to induction is that the activation of *FT* in the leaves results in the production of a secondary signal that moves to the meristem where it induces *FT* expression. This multi-step regulation/signalling process might be required to fine-tune the flowering signal at the apex to prevent flowering in non-optimal conditions.

FT as the floral stimulus

FT is a direct target of *CO* that is expressed in the leaves and not in the SAM, but FT acts in the meristem to regulate gene expression suggesting that *FT* mRNA or protein moves to the SAM. Recent work suggests that *FT* mRNA might be the moving signal. Using a heat-shock inducible promoter fused to *FT* (*Hsp::FT*), Huang *et al*. (2005) showed that a single burst of *FT* expression in a single leaf of SD grown plants was able to trigger flowering in *Arabidopsis*, strongly supporting the major role played by *FT* in the leaf in the control of flowering time. Interestingly, the treated leaf could be removed from the plant 7 h after the heat treatment suggesting that the *FT*-dependent signal had left the leaf within this time window. This result gives some indication of the timing of the movement of the floral stimulus out of the leaf in *Arabidopsis* and is compatible with the results of Corbesier *et al*. (1996) showing that the slowest component of the stimulus started to be exported from the leaves between 8 h and 12 h after the shift from SD to LD. Interestingly, in *Lolium temulentum*, King *et al*. (2006) also observed a dramatic increase in the level of *LtFT* mRNA in the leaves within 16 h of exposure to the critical daylength for flowering suggesting that *LtFT* RNA or protein could be part of the floral stimulus together with GAs in that species.

Later, Huang *et al*. (2005) detected *Hsp::FT* mRNA in the apex of *Arabidopsis* plants (8-fold induction in the apex compared with 2000-fold in the leaf) and this increase occurred 24 h after the treatment while the increased *Hsp::FT* mRNA level stopped after 3 h in the heat shock-treated leaf. In addition, 9 h after the end of the *Hsp::FT* mRNA burst, a strong increase of the endogenous *FT* mRNA occurred in the leaf and, at the same time in the apex. This 9 h lag phase suggests that *FT* does not directly stimulate its own transcription but intermediary factors might be implicated. Interestingly, the observed continuous increase in endogenous *FT* mRNA would suggest that *Arabidopsis*, like some other species, once induced continues to produce the flowering signals which might be represented here by *FT* mRNA. In *Perilla*, for example, once induced, a single leaf stably produces the stimulus, and can induce flowering in multiple shoots; repeated grafting of a single induced *Perilla* leaf sequentially triggered flowering in seven shoots over a period of 97 d (Zeevaart, 1985). In *Xanthium*, *Silene armeria*, and *Bryophyllum daigrementianum* shoots induced to flower by grafting to donor shoots can themselves act as donors in

subsequent grafts (Zeevaart, 1976). This suggests that the floral stimulus can act in the leaves of these species to trigger its own synthesis. However, this phenomenon may not be widespread, since other species, such as *Perilla*, do not exhibit indirect induction of flowering. On the other hand, in *Arabidopsis*, the fact that leaves can be removed from plants once the stimulus has been emitted (Corbesier *et al*., 1996; Huang *et al*., 2005) suggests that this species does not need the continuous synthesis of the stimulus and thus, it can be hypothesized that the increase of endogenous *FT* mRNA expression found by Huang *et al*. (2005) late after the end of the heat treatment may provide a mechanism for maintaining the induced state.

On the other hand, FT is a small protein of 23 kDa (Kardailsky *et al*., 1999; Kobayashi *et al*., 1999) and thus is below the size exclusion limit of plasmodesmata (Imlau, 1999). This small size suggests that the FT protein may move freely through plant tissues. Symplastic downloading of proteins from the sieve elements into the sink tissues of the apex through plasmodesmata has been proposed (Ruiz-Medrano *et al*., 2001), suggesting that FT may move directly by this mechanism into apical cells and induce flowering. However, so far, the movement of FT protein during the floral transition has not been demonstrated, but FT protein was found in the proteome of the phloem sap of *Brassica napus* collected from the inflorescences of plants 1 week after the flower buds become visible by eye (Giavalisco *et al*., 2006). Based on high resolution 2-D gel electrophoresis, they were able to detect 600 spots among which 140 could be reliably identified by MALDI-MS peptide fingerprints or by partial sequence determination by mass spectrometry. Among these 140 proteins, they identified both the FT and the homolog of FT, TWIN SISTER OF FT (TSF), proteins. This paper is the first published work showing that the FT protein can indeed be detected in the phloem sap. However, the movement of the FT protein was not linked to the floral transition since the sap was collected on plants which had already formed floral buds. The proteome analysed is most probably linked with the process of inflorescence/flower development. This is supported by the presence of latex proteins in the sap, some of which are involved in fruit ripening in melon (Aggelis *et al*., 1997) High-resolution phloem protein profiling is difficult to establish in *Arabidopsis* because of its rosette habit and should be used in other species able to be induced by a photoperiodic treatment allowing a synchronous shift from the vegetative stage to flowering. Such ideal species would possess a stem at the vegetative stage and belong to the same family as *Arabidopsis* to render possible the sequence-based identification of proteins. Although the genetic study of the flowering process is still incomplete in this species, *Sinapis* could be considered a species of choice for this type of work since the movement of the floral stimulus out of the leaves has been precisely timed in this caulescent species (Bernier *et al*., 1993). So far, whether the *FT* mRNA, the FT protein or both move in wild-type plants remains to be established, as does the requirement of any movement for flowering.

In agreement with the latter hypothesis, Lifschitz *et al*. (2006) recently identified the tomato *FT* orthologue as *SINGLE-FLOWER TRUSS* (*SFT*), a gene regulating primary shoot flowering time, sympodial habit, and flower morphology (Carmel-Goren *et al*. 2003). *sft* mutants showed late flowering, indeterminate vegetative inflorescence shoots with few flowers each with a single enlarged sepal. Constitutive expression of *SFT* under the *35S* promoter induced extreme early flowering in day-neutral tobacco and tomato. In addition, when *sft* receptor shoots were grafted on *35S::SFT* donors, the receptor shoots produced normal flowers, normal inflorescences and normal sympodial architecture suggesting that graft transmissible signals initiated by the *SFT* gene rescued flowering time and morphogenesis defects in *sft* mutants. Interestingly, graft-transmissible *SFT* signals also substituted for the long-day stimuli in *Arabidopsis* when expressed under the control of a leaf-specific promoter, short-day stimuli in Maryland Mammoth tobacco, and light-dose requirements in tomato *uniflora* mutant plants. In tomato, *SFT* is expressed in the leaf veins, shoot apices, stem, but not in roots, nor in the SAM itself where the floral transition takes place. A G-box factor called *SPGB* was also detected in the leaves of tomato and the closest homologue of that gene in *Arabidopsis* is *FD* suggesting a role for *SFT* in the leaves directly, potentially making it unnecessary for *SFT* RNA to travel toward its interacting partners as implied for *Arabidopsis*. The localization of *SFT* RNA was performed on grafted flowering plants and while they were able to detect the RNA in the donor leaves, the authors could not detect it in the receiver apices suggesting that florigen-like messages in tomato are part of a downstream pathway triggered by cell-autonomous *SFT* RNA transcripts. This is in contrast to the conclusion that *FT* mRNA comprises a transmissible signal in *Arabidopsis*.

Besides its role in the SAM, FT *also regulates gene expression in the leaves*

While it is clear that *FT* acts, together with *FD*, in the SAM to up-regulate *SOC1* and *AP1*, Teper-Bamnolker and Samach (2005) give some support for a role of *FT* directly in the leaves. Normally, early flowering is associated with small leaves. An association between ontogenetic changes in vegetative metamers (heteroblasty) during plant development and the transition to the reproductive stage was noted and documented a century ago, but the link between these two processes remained unclear (Goebel, 1900; Jones, 1999). In *Arabidopsis*, LD reduce both flowering time and rosette leaf size. Most late-flowering mutants, including *ft* and *fd* loss-of-function mutants, have larger rosette leaves. In early-flowering ecotypes exposed to LDs, the

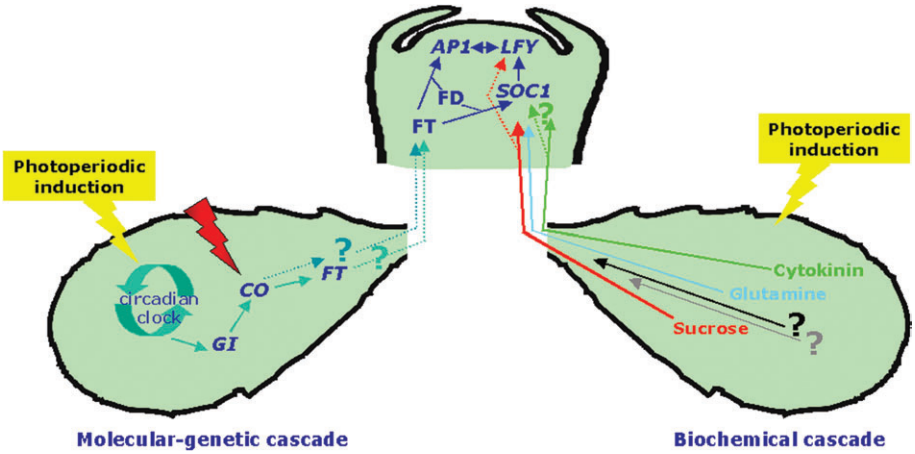

Fig. 2. Signalling cascades regulating flowering time by photoperiod in *Arabidopsis*. On the left is the molecular-genetic cascade involving transcriptional activation of genes such as *CO* and *FT* in the leaves through the circadian clock in response to LDs. The result of this gene activation is then transmitted to the SAM where floral morphogenesis takes place. How this signal is transmitted remains unclear, but it might involve movement of a product of *FT*. On the right is the biochemical cascade in which LD induction causes increased export of sucrose, glutamine, and cytokinins from the leaves towards the SAM. Both the molecular and biochemical changes occurring in the leaves in response to LDs activate a second molecular cascade in the SAM leading to *SOC1* expression and finally to the activation of *LFY* and *AP1*, which induce floral morphogenesis in the SAM. The major unresolved question is how these molecular and biochemical changes interact with each other, both in the leaves and in the SAM. Figure adapted from Corbesier and Coupland (2005): Corbesier L, Coupland G. 2005. Photoperiodic flowering of *Arabidopsis*: integrating genetic and physiological approaches to characterization of the floral stimulus. *Plant, Cell and Environment* **28,** 54–66, and reproduced by kind permission of Blackwell Publishing.

fate of existing leaf primordia is changed with the transition to flowering (Hempel and Feldman, 1994). It appears as if a signal is sent downward from the SAM to leaf primordia, and this message is diluted with distance or with the developmental stage of the leaf, so that it only affects leaves at a certain stage. As a result, one can observe basipetal (top to base) initiation of inflorescence shoots from the axils of these leaves that have acquired a cauline fate (Hempel and Feldman, 1994). Teper-Bamnolker and Samach (2005) suggest that *FT* could be responsible for the onset of this signal.

These authors observed that overexpression of *FT*, besides causing early flowering, alters the morphology of leaves: *35S::FT* plants showed a reduction in leaf size and leaf curling. In addition, expression of *SEP3* and *FUL*, which encode MADS box proteins, was increased in those leaves, and reduced in the rosette leaves of the *ft-2* mutant as well as in a *fd-1* mutant. Interestingly, overexpression of *FT* in the *fd-1* mutant, had little effect on flowering time but suppressed leaf curling and leaf size reduction suggesting that leaf fate might be regulated by *FT* in a *FD*-dependent manner via the up-regulation of *FUL*. The role of FUL was supported by the observation that the *ful* mutation suppressed the leaf and flowering-time phenotypes of the *35S::FT* plants.

Thus *FT* promotes flowering in the SAM by affecting transcription of selected transcription factors that together initiate a cascade of events leading to *FT*-dependent transcriptional changes in hundreds of genes within the apex (Schmid *et al*., 2003). These results of Teper-Bamnolker and Samach (2005) show that *FT* could regulate, through *SEP3* and *FUL*, the fate of leaf primordia during the transition to flowering, by reducing the size of cauline leaves.

Vernalization also regulates FT *expression in* Arabidopsis

Classical physiological experiments suggested that vernalization, which is the promotion of flowering by extended exposures to low temperatures that mimic winter conditions, acts in the SAM to promote flowering (Michaels and Amasino, 2000). Initial observations were based on localized cooling of shoot apices, which include young leaf primordia as well as the SAM, and this was sufficient for vernalization of celery, chrysanthemum, and *Thlaspi arvense* (Curtis and Chang, 1930; Schwabe, 1954; Metzger, 1988). However, these studies cannot distinguish between cold responses at the meristem and young leaves. Further evidence indicates that vernalization can indeed also occur in leaves: young leaves of sugar beet plants were induced to produce the floral stimulus by vernalization and flowering plants were regenerated from vernalized leaves of *Luannari biennis* and *Thlaspi*, but not from non-vernalized leaves (Wellensiek, 1964; Metzger, 1988; Crosthwaite and Jenkins, 1993).

In *Arabidopsis*, a major response to extended exposure to low temperature is the reduction of abundance of the mRNA encoding the MADS box transcription factor *FLOWERING LOCUS C* (*FLC*), which is a potent repressor of flowering (Michaels and Amasino, 1999*a*, *b*; Sheldon *et al*., 1999) (Fig. 1). *FLC* is expressed widely in the plant including the SAM, the root meristem, and the leaves making it difficult to identify the tissues in which

FLC expression is required to repress flowering (Michaels and Amasino, 2000; Sheldon *et al*., 2002; Noh and Amasino, 2003; Bastow *et al*., 2004). However, since the expression of *FT* is reduced by *FLC* and *FT* is an early target of *CO* which has been shown to act in the leaf vasculature (Takada and Goto, 2003; An *et al*., 2004; Ayre and Turgeon, 2004), it is likely that *FLC* has a role in expanded leaves.

Further support to the hypothesis that *FLC* plays a role in flowering time control in the SAM and leaves came from misexpressing the gene in specific organs (Searle *et al*., 2006). *FLC* expression in the phloem through both the *Agrobacterium rhizogenes ROLC* (Guivarc'H *et al*., 1996) and *SUC2* promoters in *flc-3* mutants caused late flowering. A similar delay in flowering was also observed when *FLC* expression was driven in the SAM through the promoter of the Knotted-like from *Arabidopsis thaliana* 1 gene (*KNAT1*; Lincoln *et al*., 1994). Interestingly, an enhanced delay in flowering time was observed by combining phloem and SAM expression of *FLC*, suggesting that it acts both in the leaves and meristem to cause the severe delay in flowering observed in winter-annual accessions.

Using chromatin immunoprecipitation experiments, Searle *et al*. (2006) showed that, prior to vernalization, FLC binds directly to *FT* and this prevents the formation of a systemic signal that would otherwise activate expression of *SOC1* at the SAM. In addition, FLC suppresses the response to the *FT*-dependent signal produced in the leaves by directly binding to *SOC1* and *FD* promoters and reducing their expression in the meristem. This activity in the meristem reduces the competence of the SAM to respond to systemic signals formed in the leaf. The authors thus demonstrate that, in *Arabidopsis*, vernalization, by repressing *FLC*, acts both in the leaves and in the SAM. The FT-based systemic signal produced in the leaves is therefore also controlled by FLC and vernalization in winter-annual accessions of *Arabidopsis*.

The same conclusion was previously reached by Michaels *et al*. (2005) who showed that activation of *FT* and *TSF* strongly suppressed the FLC-mediated late flowering phenotype of winter annuals without affecting *FLC* mRNA levels. Rather, they observed that *FT* and *TSF* bypassed the block of flowering created by *FLC* by directly activating *SOC1* expression. They suggested that the integration of flowering signals from the photoperiod and vernalization pathways occurs, in part, through the regulation of *FT*, *TSF*, and *SOC1* in winter-annual *Arabidopsis* accessions.

Prospects

Major goals in understanding the photoperiodic control of flowering are to link the activation of flowering-time genes expressed in leaves with the export of the floral signal; to analyse the roles played by these signals at the SAM; and to identify the systemic signals involved. Despite recent progress in understanding the mode of action of *FT* in the SAM, the nature of the transmitted signal and the significance of transport of *FT* mRNA still remain unclear (Fig. 2). Nevertheless, analysis of *FT* expression and its functions in the leaf and SAM are likely to bring us closer to the identification of the floral stimulus.

References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T. 2005. FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* **309,** 1052–1056.

Aggelis A, John I, Karvouni Z, Grierson D. 1997. Characterization of two cDNA clones for mRNAs expressed during ripening of melon (*Cucumis melo* L.) fruits. *Plant Molecular Biology* **33,** 313–322.

An H, Roussot C, Suarez-Lopez P, *et al*. 2004. CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of *Arabidopsis*. *Development* **131,** 3615–3626.

Ayre B, Turgeon R. 2004. Graft transmission of a floral stimulant derived from CONSTANS. *Plant Physiology* **135,** 2271–2278.

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C. 2004. Vernalization requires epigenetic silencing of *FLC* by histone methylation. *Nature* **427,** 164–167.

Bernier G. 1988. The control of floral evocation and morphogenesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **39,** 175–219.

Bernier G, Havelange A, Houssa C, Petitjean A, Lejeune P. 1993. Physiological signals that induce flowering. *The Plant Cell* **5,** 1147–1155.

Bernier G, Périlleux C. 2005. A physiological overview of the genetics of flowering time control. *Plant Biotechnology Journal* **3,** 3–16.

Blazquez MA, Ahn JH, Weigel D. 2003. A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. *Nature Genetics* **33,** 168–171.

Borner R, Kampmann G, Chandler J, Gleissner R, Wisman E, Apel K, Melzer S. 2000. A MADS domain gene involved in the transition to flowering in *Arabidopsis*. *The Plant Journal* **24,** 591–599.

Boss PK, Bastow RM, Mylne JS, Dean C. 2004. Multiple pathways in the decision to flower: enabling, promoting, and resetting. *The Plant Cell* **16,** S18–S31.

Carmel-Goren L, Liu TS, Lifschitz E, Zamir D. 2003. The *SELF-PRUNING* gene family in tomato. *Plant Molecular Biology* **52,** 1215–1222.

Cerdan PD, Chory J. 2003. Regulation of flowering time by light quality. *Nature* **423,** 881–885.

Chailakhyan MK. 1937. Concerning the hormonal nature of plant development processes. *Doklady Akad Nauk SSSR* **16,** 227– 230.

Corbesier L, Coupland G. 2005. Photoperiodic flowering of *Arabidopsis*: integrating genetic and physiological approaches to characterization of the floral stimulus. *Plant, Cell and Environment* **28,** 54–66.

Corbesier L, Gadisseur I, Silvestre G, Jacqmard A, Bernier G. 1996. Design in *Arabidopsis thaliana* of a synchronous system of floral induction by one long day. *The Plant Journal* **9,** 947–952.

Corbesier L, Kustermans G, Périlleux C, Melzer S, Moritz T, Havelange A, Bernier G. 2004. Gibberellins and the floral transition in Sinapis alba. *Physiologia Plantarum* **122,** 152–158.

Crosthwaite SK, Jenkins GI. 1993. The role of leaves in the perception of vernalizing temperatures in sugar-beet. *Journal of Experimental Botany* **44,** 801–806.

Curtis OF, Chang HT. 1930. The relative effectiveness of the temperature of the crown as contrasted with that of the rest of the plant upon the flowering of celery plants. *American Journal of Botany* **17,** 1047–1048.

Evans LT. 1969. The nature of flower induction. In: Evans LT, ed. *The induction of flowering: some case histories*. Melbourne: The Macmillan Company of Australia, 457–480.

Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Coupland G, Putterill J. 1999. *GIGANTEA*: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. *EMBO Journal* **18,** 4679–4688.

Giavalisco P, Kapitza K, Kolasa A, Buhtz A, Kehr J. 2006. Towards the proteome of *Brassica napus* phloem sap. *Proteomics* **6,** 896–909.

Guivarc' HA, Spena A, Noin M, Besnard C, Chriqui D. 1996. The pleiotropic effects induced by the *rolC* gene in transgenic plants are caused by expression restricted to protophloem and companion cells. *Transgenic Research* **5,** 3–11.

Goebel K. 1900. *Organography of plants*. I. *General organography*. New York: Hafner.

Hastings MH, Follet BK. 2001. Towards a molecular biological calendar? *Journal of Biological Rhythms* **15,** 424–430.

Hempel FD, Feldman LJ. 1994. Bi-directional inflorescence development in *Arabidopsis thaliana*: acropetal initiation of flowers and basipetal initiation of paraclades. *Planta* **192,** 276–286.

Huang T, Bohlenius H, Eriksson S, Parcy F, Nilsson O. 2005. The mRNA of the *Arabidopsis* gene *FT* moves from leaf to shoot apex and induces flowering. *Science* **309,** 1694–1696.

Imlau A, Truernit E, Sauer N. 1999. Cell-to-cell and long-distance trafficking of the green fluorescent protein in the phloem and symplastic unloading of the protein into sink tissues. *The Plant Cell* **11,** 309–322.

Jack T. 2004. Molecular and genetic mechanisms of floral control. *The Plant Cell* **S16,** 1–17.

Jones CS. 1999. An essay on juvenility, phase change, and heteroblasty in seed plants. *International Journal of Plant Science* **160,** S105–S111.

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D. 1999. Activation tagging of the floral inducer *FT*. *Science* **286,** 1962–1965.

King RW, Evans LT, Wardlaw IF. 1968. Translocation of the floral stimulus in *Pharbitis nil* in relation to that of assimilates. *Zeitschrift für Pflanzenphysiologie* **59,** 377–388.

King RW, Moritz T, Evans LT, Junttila O, Herlt AJ. 2001. Long-day induction of flowering in *Lolium temulentum* involves sequential increases in specific gibberellins at the shoot apex. *Plant Physiology* **127,** 624–632.

King RW, Moritz T, Evans LT, Martin J, Andersen CH, Blundell C, Kardailsky I, Chandler PM. 2006. Regulation of flowering in the long day grass, *Lolium temulentum* L., by gibberellins and the gene *FLOWERING LOCUS T (FT)*. *Plant Physiology* **141,** 498–507.

King RW, Zeevaart JAD. 1973. Floral stimulus movement in *Perilla* and flower inhibition caused by noninduced leaves. *Plant Physiology* **51,** 727–738.

Knott JE. 1934. Effect of a localized photoperiod on spinach. *Proceedings of the Society for Horticultural Science* **31,** 152–154.

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T. 1999. A pair of related genes with antagonistic roles in mediating flowering signals. *Science* **286,** 1960–1962.

Koornneef M, Hanhart CJ, Van Der Veen JH. 1991. A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Molecular and General Genetics* **229,** 57–66.

Lee H, Suh S-S, Park E, Cho E, Ahn JH, Kim S-G, Lee JS, Kwon YM, Lee I. 2000. The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. *Genes and Development* **14,** 2366–2376.

Lifschitz E, Eviatar T, Rozman A, Shalit A, Goldshmidt A, Amsellem Z, Alvarez JP, Eshed Y. 2006. The tomato *FT* ortholog triggers systemic signals that regulate growth and flowering and substitute for divers environmental stimuli. *Proceedings of the National Academy of Sciences, USA* **103,** 6398–6403.

Lincoln C, Long J, Yamaguchi J, Serikawa K, Hake S. 1994. A knotted1-like homeobox gene in *Arabidopsis* is expressed in the vegetative meristem and dramatically alters leaf morphology when overexpressed in transgenic plants. *The Plant Cell* **6,** 1859–1876.

Metzger JD. 1988. Localization of the site of perception of thermoinductive temperatures in *Thlaspi arvense* L. *Plant Physiology* **88,** 424–428.

Michaels SD, Amasino RM. 1999a. *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *The Plant Cell* **11,** 949–956.

Michaels SD, Amasino RM. 1999b. The gibberellic acid biosynthesis mutant *ga1-3* of *Arabidopsis thaliana* is responsive to vernalization. *Developmental Genetics* **25,** 194–198.

Michaels SD, Amasino RM. 2000. Memories of winter: vernalization and the competence to flower. *Plant. Cell and Environment* **23,** 1145–1153.

Michaels SD, Himelblau E, Kim SY, Schomburg FM, Amasino RM. 2005. Integration of flowering signals in winter-annual *Arabidopsis*. *Plant Physiology* **137,** 149–156.

Mizoguchi T, Wright L, Fujiwara S, *et al*. 2005. Distinct roles of *GIGANTEA* in promoting flowering and regulating circadian rhythms in *Arabidopsis*. *The Plant Cell* **17,** 2255–2270.

Moon J, Suh SS, Lee H, Choi KR, Hong CB, Paek NC, Kim SG, Lee I. 2003. The *SOC1* MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. *The Plant Journal* **35,** 613–623.

Mouradov A, Cremer F, Coupland G. 2002. Control of flowering time: interacting pathways as a basis for diversity. *The Plant Cell* **14,** S111–S130.

Noh YS, Amasino RM. 2003. *PIE1*, an ISWI family gene, is required for *FLC* activation and floral repression in *Arabidopsis*. *The Plant Cell* **15,** 1671–1682.

Page T, Macknight R, Yang CH, Dean C. 1999. Genetic interactions of the *Arabidopsis* flowering time gene *FCA*, with genes regulating floral initiation. *The Plant Journal* **17,** 231–239.

Park DH, Somers DE, Kim YS, Choy YH, Lim HK, Soh MS, Kim HJ, Kay SA, Nam HG. 1999. Control of circadian rhythms and photoperiodic flowering by the *GIGANTEA* gene. *Science* **285,** 1579–1582.

Pineiro M, Gomez-Mena C, Schaffer R, Martinez-Zapater JM, Coupland G. 2003. *EARLY BOLTING IN SHORT DAYS* is related to chromatin remodelling factors and regulates flowering in *Arabidopsis* by repressing *FT*. *The Plant Cell* **15,** 1552–1562.

Putteril J, Laurie R, Macknight R. 2004. It's time to flower: the genetic control of flowering time. *Bioessays* **26,** 363–373.

Putterill J, Robson F, Lee K, Simon R, Coupland G. 1995. The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes

a protein showing similarities to zinc finger transcription factors. *Cell* **80,** 847–857.

Redei GP. 1962. Supervital mutants of *Arabidopsis*. *Genetics* **47,** 443–460.

Robson F, Costa MMR, Hepworth S, Vizir I, Pineiro M, Reeves PH, Putterill J, Coupland G. 2001. Functional importance of conserved domains in the flowering-time gene *CONSTANS* demonstrated by analysis of mutant alleles and transgenic plants. *The Plant Journal* **28,** 619–631.

Ruiz-Garcia L, Madueno F, Wilkinson M, Haughn G, Salinas J, Martinez-Zapater JM. 1997. Different roles of flowering-time genes in the activation of floral initiation genes in *Arabidopsis*. *The Plant Cell* **9,** 1921–1934.

Ruiz-Medrano R, Xoconostle-Cazares B, Lucas WJ. 2001. The phloem as a conduit for inter-organ communication. *Current Opinion in Plant Biology* **4,** 202–209.

Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G. 2000. Distinct roles of *CONSTANS* target genes in reproductive development of *Arabidopsis*. *Science* **288,** 1613–1616.

Schmid M, Uhlenhaut NH, Godard F, Demar M, Bressan R, Weigel D, Lohmann JU. 2003. Dissection of floral induction pathways using global expression analysis. *Development* **130,** 6001–6012.

Schwabe WW. 1954. Factors affecting flowering in *Chrysanthemum*. IV. The site of vernalization and translocation of the stimulus. *Journal of Experimental Botany* **5,** 389–400.

Searle I, Coupland G. 2004. Induction of flowering by seasonal changes in photoperiod. *EMBO Journal* **23,** 1217–1222.

Searle I, He Y, Turck F, Vincent C, Fornara F, Krober S, Amasino RA, Coupland G. 2006. The transcription factor *FLC* confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis*. *Genes and Development* **20,** 898–912.

Sheldon CC, Conn AB, Dennis ES, Peacock WJ. 2002. Different regulatory regions are required for the vernalization-induced repression of *FLOWERING LOCUS C* and for the epigenetic maintenance of repression. *The Plant Cell* **14,** 2527–2537.

Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES. 1999. The *FLF* MADS box gene: a repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. *The Plant Cell* **11,** 445–458.

Simon R, Igeno MI, Coupland G. 1996. Activation of floral meristem identity genes in *Arabidopsis*. *Nature* **384,** 59–62.

Suarez-Lopez P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G. 2001. *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature* **410,** 1116–1120.

Takada S, Goto K. 2003. TERMINAL FLOWER2, an *Arabidopsis* homolog of HETEROCHROMATIN PROTEIN1, counteracts the activation of *FLOWERING LOCUS T* by CONSTANS in the vascular tissues of leaves to regulate flowering time. *The Plant Cell* **15,** 2856–2865.

Teper-Bamnolker P, Samach A. 2005. The flowering integrator *FT* regulates *SEPALLATA3* and *FRUITFULL* accumulation in *Arabidopsis* leaves. *The Plant Cell* **17,** 2661–2675.

Truernit E, Sauer N. 1995. The promoter of the *Arabidopsis thaliana SUC2* sucrose-H^+ symporter gene directs expression of β-glucuronidase to the phloem: evidence for phloem loading and unloading by SUC2. *Planta* **196,** 564–570.

Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G. 2004. Photoreceptor regulation of CONSTANS protein and the mechanism of photoperiodic flowering. *Science* **303,** 1003–1006.

Wellensiek SJ. 1964. Dividing cells as prerequisite for vernalization. *Plant Physiology* **39,** 832–835.

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D. 2005. Integration of spatial and temporal information during floral induction in *Arabidopsis*. *Science* **309,** 1056–1059.

Yanovsky MJ, Kay SA. 2002. Molecular basis of seasonal time measurement in *Arabidopsis*. *Nature* **419,** 308–312.

Yanovsky MJ, Kay SA. 2003. Living by the calendar: how plants know when to flower. *Nature Reviews Molecular Cell Biology* **4,** 265–275.

Yoo SK, Chung KS, Kim J, Lee JH, Hong SM, Yoo SJ, Yoo SY, Lee JS, Ahn JH. 2005. CONSTANS activates *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* through *FLOWERING LOCUS T* to promote flowering in *Arabidopsis*. *Plant Physiology* **139,** 770–778.

Zeevaart JAD. 1976. Physiology of flower formation. *Annual Review of Plant Physiology* **27,** 321–348.

Zeevaart JAD. 1985. Perilla. In: Halevy AH, ed. *CRC handbook of flowering,* Vol. 5. Boca Raton, Florida: CRC Press, 239–252.