The quest for florigen: a review of recent progress

Laurent Corbesier and George Coupland*
Max Planck Institute for Plant Breeding Research, Carl von Linne Weg 10, D-50829 Cologne, Germany

Received 20 March 2006; Accepted 19 June 2006

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
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), hypoxia, 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 of the Arabidopsis 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 (Truenit 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 ft 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 daigremontianum 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 cauline 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 SPF 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 *API*, Teper-Bammolker 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
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, 1999a, 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 occurred 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**


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.


