**Int. J. Dev. Biol. 49**: 585-593 (2005)
doi: 10.1387/ijdb.041930fp

# Flowering: a time for integration

FRANÇOIS PARCY*

*Physiologie Cellulaire Végétale. CNRS UMR5168- CEA- INRA- UJF, Grenoble, France*

**ABSTRACT Flowering time is under the control of multiple environmental cues such as photoperiod and exposure to cold temperatures (vernalization). A few regulators named integrators of flowering time signals (*LEAFY, SOC1 / AGL20* and *FT*) integrate inputs from the different flowering cascades and convey the resulting outcome to floral meristem identity genes at the shoot apex. Here we review the current knowledge about the expression of integrators, their mode of action, their potential target genes and the nature of their mutual interactions. We emphasize the questions that have been generated by recent progress in this field and that remain to be addressed.**

KEY WORDS: *flowering, floral pathway integrator, LEAFY, SOC1, FT*

*"Get yourselves organized down there"*
Wallace talking to the sheep in
*A close shave* by Nick Park

## Introduction

As for other plants, the floral transition is one the most drastic changes occurring during *Arabidopsis thaliana* life cycle. The shoot apical meristem switches from the production of leaves with associated secondary shoot meristems to bractless flowers (Hempel and Feldman, 1993, Suh *et al.*, 2003). This transition is abrupt and irreversible, suggesting it is regulated by a robust gene regulatory network capable of driving sharp transitions. The moment at which this transition occurs is precisely determined by environmental and endogenous signals. *Arabidopsis* flowers earlier in long than in short days (it is a facultative long-day plant). Also, many *Arabidopsis* strains flower earlier after a period of cold exposure (a treatment named vernalization). Genetic analyses identified a whole set of flowering time mutants that were subsequently assigned to four major genetic pathways according to their response to vernalization or day length (Martinez-Zapater *et al.*, 1994). The field of flowering time has been organized around these four pathways, with the photoperiod and vernalization pathways mediating the response to environmental cues and the autonomous and the gibberellin (GA) pathways acting largely independently from these external signals (Figure 1). A large number of genes acting within these pathways have been cloned and current analyses aim at understanding how they are linked to each other and how the corresponding proteins function (Amasino, 2004, Araki, 2001, Bastow and Dean, 2003, Boss *et al.*, 2004, Jack, 2004, Mouradov *et al.*, 2002, Simpson and Dean, 2002, Simpson *et al.*, 1999, Sung and Amasino, 2004a). Two genes play a prominent role at the "bottom" of these promotion cascades. The *CONSTANS (CO)* gene is probably the most downstream actor, specific for the photoperiod pathway (Figure 1) and both the light and the internal clock precisely regulate the CO protein accumulation (Valverde *et al.*, 2004). The *FLOWERING LOCUS C* (*FLC)* gene is the point of convergence of the autonomous and vernalization pathways (Figure 1). Ultimately and in part through CO and FLC, the flowering signals lead to the induction of a set of genes called floral meristem identity (FMI) genes and responsible for the fate change of the meristems emerging on the flanks of the shoot apex (Long and Barton, 2000). This group of genes includes *LEAFY* (*LFY*), *APETALA1* (*AP1)* and *CAULIFLOWER* (*CAL* ), expressed in early floral stages and responsible for their floral fate (Kieffer and Davies, 2001, Lohmann and Weigel, 2002).

Recently, three genes were shown to make the junction between the different flowering-time cascades and the FMI genes (Figure 1). These genes were named Floral Pathway Integrators because they are able to integrate a balance of stimulations originating from the different pathways and convert these heterogeneous inputs into an induction of FMI genes, thereby initiating the production of the first floral meristems (Simpson and Dean, 2002).

Several recent excellent reviews deal with the control of flowering time (Amasino, 2004, Araki, 2001, Bastow and Dean, 2003, Boss *et al.*, 2004, Jack, 2004, Mouradov *et al.*, 2002, Simpson and Dean, 2002, Simpson *et al.*, 1999, Sung and

*Abbreviations used in this paper:* AGL20, AGAMOUS-like 20; AP1, APETALA1; CAL, CAULIFLOWER; CO, CONSTANS; FLC, FLOWERING LOCUS C; FT, FLOWERING LOCUS T; GA, gibberellins; GFP, green fluorescent protein; LD, long days; LFY, LEAFY; SD, short days; SOC1, SUPPRESSOR OF CONSTANS OVEREXPRESSION; WUS, WUSCHEL.

***Address correspondence to:** Dr. François Parcy. PCV/DRDC Bat. C2. 17, avenue des martyrs, 38054 Grenoble, cedex 9, France.
Fax +33-4-3878-5091. e-mail: francois.parcy@cea.fr

0214-6282/2005/$25.00

Printed in Spain
www.intjdevbiol.com

Amasino, 2004a). In this paper, I deliberately chose to focus on *Arabidopsis* Floral Pathway Integrators (thereafter named integrators) and to review and question in some detail the available experimental evidence subtending current models of gene regulatory network leading to flowering.

## Identity of floral pathway integrators

The three genes shown to integrate the influence from different pathways are *LEAFY(LFY)*, *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF CO OVEREXPRESSION (SOC1) /AGAMOUS-like 20 (AGL20) (* thereafter named *SOC1* ). I will first introduce these genes and their expression and then focus on their regulation by the pathways promoting flowering.

### *LEAFY (LFY)*

The *LEAFY* gene plays a key role during flower development and can be considered both as a flowering time gene and a meristem identity gene.

*LEAFY* expression precedes the floral transition: it is first detected (both the RNA and the promoter activity) in young leaf primordia and it increases to reach a maximum in young floral meristems (Blazquez *et al.*, 1998, Blazquez *et al.*, 1997) (Figure 2). Plants with increased *LFY* gene copy number or *LFY* constitutive expression flower early (Blazquez *et al.*, 1997, Weigel and Nilsson, 1995) whereas plants with a mutant *LFY* gene bear leaves and associated shoot instead of flowers (Weigel *et al.*, 1992) showing that LFY participates in determining the flowering time. Flower-like structures eventually appear on a *lfy* "inflorescence" because the FMI gene *APETALA1* can be activated in an LFY-independent manner and partially compensates for the lack of *LFY* (Bowman *et al.*, 1993, Huala and Sussex, 1992, Weigel and Meyerowitz, 1994).

LFY expression persists throughout young floral meristems where it contributes to the specification of the young floral buds by, for instance, inducing the *AP1* and *CAL* (Parcy *et al.*, 1998, Wagner and Meyerowitz, 2002, Wagner *et al.*, 2004), to the floral meristem patterning (Lohmann *et al.*, 2001, Parcy *et al.*, 1998) and to the repression of shoot identity (Parcy *et al.*, 2002, Yu *et al.*, 2004).

*LFY* encodes a new type of plant specific transcription factor (Parcy *et al.*, 1998, Weigel *et al.*, 1992). The LFY protein appears to be localized primarily in the nucleus (Parcy *et al.*, 1998, Wagner *et al.*, 1999, Wu *et al.*, 2003) but LFY-GFP fusion proteins also accumulate in the cytoplasm and the plasmodesmatal pit (Wu *et al.*, 2003). LFY is able to travel from one cell to another through plasmodesmata but the functional importance of this movement awaits confirmation (Sessions *et al.*, 2000). The LFY protein has been shown to bind cis-elements present in *AP1* and *AGAMOUS (AG)* regulatory sequences (Busch *et al.*, 1999, Lohmann *et al.*, 2001, Parcy *et al.*, 1998). LFY activity appears to be modulated by day-length since plants constitutively expressing *LFY* flower later in short days (SD) than in long days (LD), but how photoperiod affects *LFY* is still unknown (Nilsson *et al.*, 1998, Weigel and Nilsson, 1995).

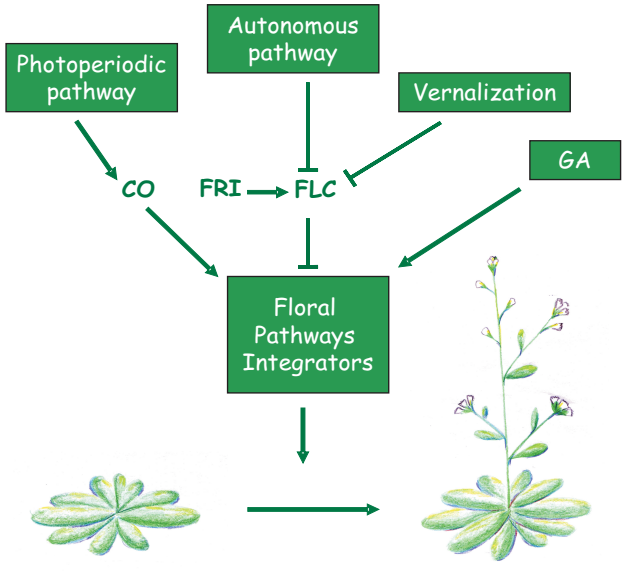


**Fig. 1. Main pathways reaching Floral Pathways Integrators**

### *Flowering locus T (FT)*

The *FT* gene was simultaneously isolated using an early-flowering activation-tagged allele and a late-flowering T-DNA mutant (Kardailsky *et al.*, 1999, Kobayashi *et al.*, 1999). The *ft* mutant is late in LD conditions and only slightly affected in SD, indicating that FT belongs to the photoperiod pathway (Koornneef *et al.*, 1991). As opposed to other flowering time mutations, the *ft* mutation strongly enhances the *lfy* mutant phenotype (Ruiz-Garcia *et al.*, 1997) and efficiently suppresses the *35S::LFY* early flowering phenotype (Nilsson *et al.*, 1998). For this reason, *FT* was assigned to a separate branch of the photoperiod pathway together with *FWA* and *FD*. *FT* encodes a protein with similarities to Phosphatidylethanolamine binding protein (PEBP) and Raf kinase inhibitor protein (RKIP) in animals (Kardailsky *et al.*, 1999, Kobayashi *et al.*, 1999), but its function in plants remains to be identified. Using the sensitive RT-PCR technique, *FT* expression was detected in all organs (Kardailsky *et al.*, 1999, Kobayashi *et al.*, 1999). Remarkably, *FT* mRNA levels in seedlings increases during vegetative growth in LD, reaching its maximum around the floral transition. *FT* expression is reduced in SD but still shows a clear increase with time. Low expression levels have precluded a precise analysis of *FT* expression pattern by *in situ* hybridization. The *FT::GUS* reporter gene shows expression primarily in phloem cells of the leaves and shoot (Takada and Goto, 2003). *FT* constitutive expression is very potent at accelerating flowering both in LD and SD. The fastest flowering is obtained by combining constitutive expression of *FT* and *LFY*: plants produce flowers after forming only one or two leaves in LD (Kardailsky *et al.*, 1999, Kobayashi *et al.*, 1999).

### *Suppressor of CO overexpression (SOC1) /AGAMOUS-like 20 (AGL20)*

*SOC1/AGL20* encodes a MADS box transcription factor. Surprisingly, the *soc1* mutant was not isolated in standard genetic screens for late flowering mutants. It came out independently in a screen for suppressor of *CONSTANS* overexpression (Onouchi *et al.*, 2000, Samach *et al.*, 2000), from an activation tagging screen in the FRIGIDA (FRI) FLC background (Lee *et al.*, 2000) and using reverse genetics (Borner *et al.*, 2000). *Soc1* mutant flowers late in both LD and SD (Borner *et al.*, 2000, Lee *et al.*, 2000). *SOC1* is

expressed mostly in leaves and in the shoot apex, its expression raises with time and a sharp increase occurs in the apex during the floral transition (Borner *et al.*, 2000, Lee *et al.*, 2000, Samach *et al.*, 2000). *SOC1* expression is absent from stage 1 flower meristem and reappears in the center of older flower meristems (Figure 2).

## Regulation of floral pathway integrator expression

LFY, SOC1 and FT have recently been referred to as Floral Pathway Integrators (Simpson and Dean, 2002) because they represent convergence nodes for several promotion pathways: the photoperiod pathway through the action of its most downstream "specific" element CO, the vernalization and autonomous pathways through the action of the FLC repressor and the GA pathway. FLC itself could also be considered as an integrator. However, we will stick to the initial definition as proposed by Simpson *et al.* (2002). It is worth pointing out here that the evidence for integrated regulation is derived essentially from integrators expression at the mRNA level. The three integrators proteins might also be regulated at many other post-transcriptional levels that have been poorly investigated so far.

### *Regulation by* CONSTANS (CO)

The *CO* gene plays a key role in the photoperiod pathway. *CO* mRNA level oscillates following a circadian rhythm and the CO protein is stabilized by light (Suarez-Lopez *et al.*, 2001, Valverde *et al.*, 2004). In LD, *CO* mRNA expression and CO protein stability coincide at dusk and the CO protein accumulates to accelerate flowering. In SD, this coincidence does not occur and the CO protein never accumulates. This mechanism elegantly explains why *co* mutant flowers late in LD but as the wild type in SD.

A whole set of data shows that CO triggers flowering by inducing the 3 integrators. *LFY* and *FT* expression are reduced in *co* mutant (Kardailsky *et al.*, 1999, Kobayashi *et al.*, 1999, Nilsson *et al.*, 1998, Suarez-Lopez *et al.*, 2001). *SOC1* expression, also, might be slightly reduced (Lee *et al.*, 2000). In addition, the late flowering phenotype of the *co* mutant is rescued by overexpression of *FT* (complete rescue), *SOC1 (* rescue almost complete*)* or *LFY* (partial rescue) (Kardailsky *et al.*, 1999, Kobayashi *et al.*, 1999, Nilsson *et al.*, 1998, Samach *et al.*, 2000). These data suggest CO positively regulates *LFY*, *FT* and *SOC1* (Figure 3). This hypothesis was largely confirmed by gain-of-function experiments. Constitutive overexpression of *CO* (or of the inducible CO-GR fusion) induces early flowering and increases the expression of *FT*, *SOC1* and *LFY* (Samach *et al.*, 2000, Simon *et al.*, 1997). Induction of *SOC1* and *FT* are rapid (a few hours) and direct (without intermediate translation step), whereas *LFY* induction takes one day suggesting that it might be more indirect (Samach *et al.*, 2000). Both *soc1* and *ft* mutations delay flowering of *35S::CO* plants confirming that CO acts through *FT* and *SOC1* induction (Onouchi *et al.*, 2000, Samach *et al.*, 2000, Suarez-Lopez *et al.*, 2001). Its worth pointing out that the expression of the 3 integrators is still upregulated in the *co* mutant, indicating that CO is no the only factor responsible for their induction.

*CO* encodes a nuclear zinc finger containing protein, a potential transcription factor, but the precise mechanism of CO action is not yet understood (Putterill *et al.*, 1995). In particular, CO has not been shown to bind DNA and is, therefore, assumed to be tethered to regulatory sequences through interaction with other (unknown)

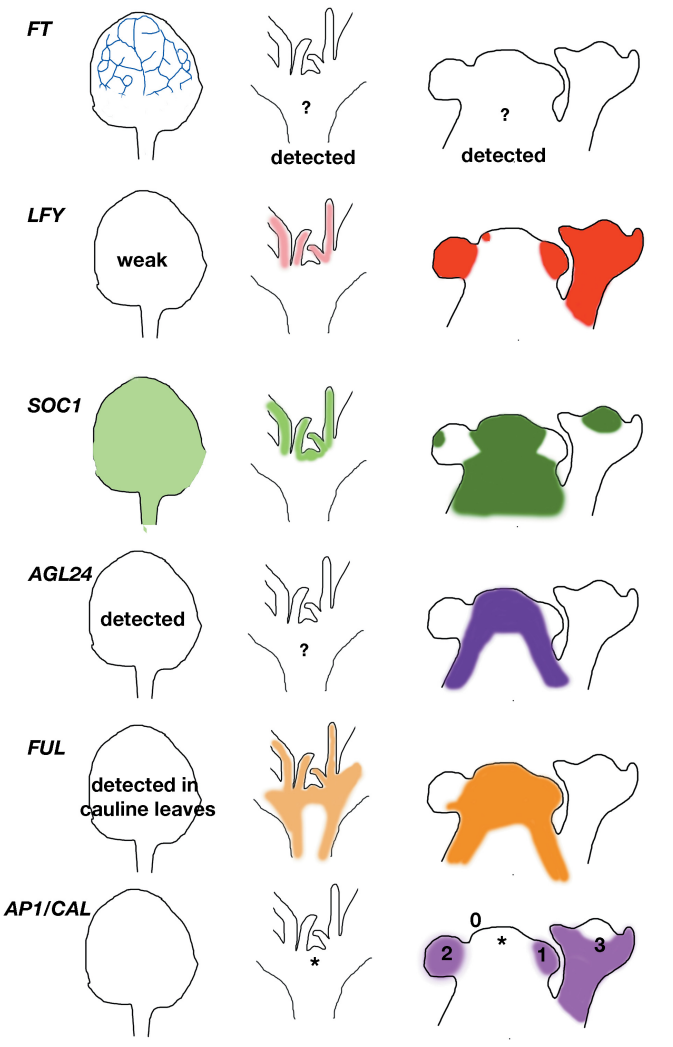


**Fig. 2. Rough expression patterns of some integrators and some other important regulators.** *A leaf, a vegetative and a reproductive apex are depicted. Color represent mRNA (or promoter::GUS) expression patterns for the genes indicated. "Detected" means the expression has been detected by RT-PCR or Northern analysis but the expression pattern is not known. A change in color brightness (as for* AGL24, SOC1 *and "*FUL *) indicates an increased expression upon floral transition. The star indicates the shoot apical meristem, the numbers indicate floral meristem stages (Smyth* et al.*, 1990).* AGL24 *expression has also been found in outer layers of early floral stages (Yu* et al.*, 2004).*

transcription factors (Hepworth *et al.*, 2002). The precise analysis of *CO* expression pattern has recently led to new and exciting questions regarding CO mode of action (An *et al.*, 2004, Takada and Goto, 2003). Indeed, the photoperiodic signal was known to be perceived in leaves and somehow transmitted to the apex by the unknown florigen signal (Bernier *et al.*, 1993, Colasanti and Sundaresan, 2000, Zeevaart, 1976). The discovery that CO is expressed in the vascular system of the leaves (in the phloem companion cells) and induces *FT* in this tissue, suggests that the florigen signal is downstream or at the same level as *CO* (An *et al.*, 2004, Takada and Goto, 2003). Expression of *CO* from different promoters showed that CO triggers early flowering when ex-

pressed in the leaf phloem but not in the apex (An *et al.*, 2004, Ayre and Turgeon, 2004). These experiments nicely suggested that CO acts from the leaves and that the florigen is downstream of *CO*. As opposed to *CO*, its target gene *FT* can trigger early flowering when expressed either from the leaves or from the apex, suggesting either that FT itself is the florigen or that FT can induce the florigen synthesis both from leaves and the apex. The possibility that CO would need to travel to the apex and be modified on its way has also been proposed (Ayre and Turgeon, 2004) but requires further investigations. Why CO cannot trigger early flowering when expressed from the apex is not understood. CO might require a leaf coregulator (maybe its DNA binding partner), absent from the apex and necessary to induce *SOC1* and *FT*. This hypothesis could be easily tested by analyzing whether the increased *FT::GUS* expression of *35S::CO* plants invades the shoot apical meristem (Takada and Goto, 2003).

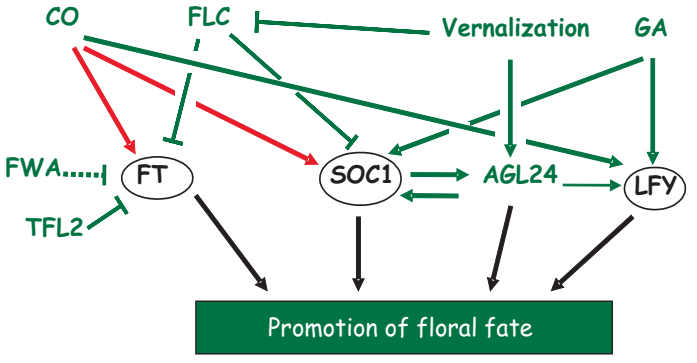


**Fig. 3. Gene regulatory network around Floral Pathway Integrators.** *Red arrows represent direct interactions, dashed arrows represent post-transcriptional regulation and plain arrows represent transcriptional regulation. Floral Pathway Integrators are circled.*

Knowing that CO acts from the leaves to induce *FT* also raises many questions about the induction of *SOC1* and *LFY*. Both *LFY* and *SOC1* expression increase at the apex during the floral transition (*SOC1* in the apex itself and *LFY* in the flower anlagen). *LFY* could be induced indirectly (for instance through *SOC1* - see later) but *SOC1* has been shown to be a direct target of CO in *35S::CO-GR* plants (Samach *et al.*, 2000). However, in most published reports, *SOC1* expression or *SOC1* promoter activation has been analyzed in whole seedlings without precise characterization of the induction in the apex (Hepworth *et al.*, 2002, Lee *et al.*, 2000). The limitation of this type of analyses appears in situations where there is no correlation between the global expression level of *SOC1* or *FT* and flowering time (Hepworth *et al.*, 2002). Precisely characterizing where does CO induce *SOC1* during the transition of wild type plants and from where *SOC1* is able to trigger flowering would be important to understand whether SOC1 also might be part of the florigen signal.

### *Regulation by FLC*

*FLC* plays a major role in repressing flowering in *Arabidopsis* (Michaels and Amasino, 1999). It is the convergence point of the autonomous and the vernalization pathways (Figure 1) and is also regulated by other genes such as *FRI*, *ELF5* or *PIE* (Michaels and Amasino, 1999, Noh and Amasino, 2003, Noh *et al.*, 2004). Differences in *FLC* and *FRI* sequence or expression account for a lot of the natural variation between *Arabidopsis* accessions (Johanson *et al.*, 2000, Michaels *et al.*, 2003b). To accelerate flowering, the autonomous and the vernalization promotion pathways repress *FLC* and maintain it in a repressed state using various epigenetic mechanisms (Bastow *et al.*, 2004, He *et al.*, 2003, Sung and Amasino, 2004b).

Several types of evidence show that FLC acts by repressing the integrators *FT* and *SOC1* : i) FLC is necessary for the downregulation of *SOC1* occurring in autonomous pathway mutants (Michaels and Amasino, 1999, Samach *et al.*, 2000). ii) *SOC1* is repressed in *35S::FLC* transgenic plants (Michaels and Amasino, 1999) iii) Both *SOC1* and *FT* are upregulated in an *flc* null mutant (Moon *et al.*, 2003). We do not know exactly from which part of the plant FLC acts. Vernalization has been shown to be perceived at the shoot apex (Amasino, 2004) and *FLC* is expressed mainly in shoot and root apices but its mRNA is also detectable in leaves (He *et al.*, 2003, Michaels and Amasino, 1999). It will be interesting to determine whether FLC represses *FT* and *SOC1* from the leaves (where CO induces *FT*) or from the apex. So far, there is no clear evidence that FLC also directly affects *LFY* expression. However, late flowering mutants of the autonomous pathway, *LFY* expression is decreased and this effect is probably mediated through FLC (Nilsson *et al.*, 1998).

*FLC* encodes a MADS-box factor and has been shown to bind to regulatory sequences of *SOC1* necessary for its repression in leaves (Hepworth *et al.*, 2002). However, as pointed out before, this repression has mainly been studied in leaves where its relevance remains to be established (Hepworth *et al.*, 2002).

### *Regulation by GA*

As attested by GA biosynthetic and response mutants, GA is crucial to promote flowering mainly in short day conditions. The *ga1* biosynthetic mutant flowers extremely late (sometimes never) in SD (Blazquez *et al.*, 1998, Wilson *et al.*, 1992). GA acts, at least in part, by upregulating'*LFY*: *LFY* expression is dramatically reduced in *ga1* mutant in SD and constitutive expression of *LFY* is sufficient to rescue the late flowering of this mutant (Blazquez *et al.*, 1998). A cis-element has been found in the *LFY* promoter that abolishes its response to GA without affecting *LFY* induction by photoperiod, indicating that the two different pathways are integrated at the level of *LFY* promoter (Blazquez and Weigel, 2000). This cis-element resembles a MYB factor binding site and interacts with the AtMYB33 protein *in vitro* (Gocal *et al.*, 2001) but the identity of the transcription factor responsible for *LFY* induction by GA remains to be firmly established.

GA is also involved in inducing *SOC1* expression (Moon *et al.*, 2003) and maybe also *FT*. This last point has not been demonstrated but, in SD, GA are required for flowering and *FT* displays a peak of expression (albeit lower as in LD), suggesting GA might be responsible for *FT* induction in these growth conditions. Also, early flowering of the *ebs* mutant is dependent on GA and EBS represses *FT*, again suggesting that GA might be responsible for FT derepression in the *ebs* mutant (Gomez-Mena *et al.*, 2001, Pineiro *et al.*, 2003).

As for CO action, it will be important to determine from where the GA signal originates and what are the steps leading to *LFY* upregulation. In short days, the concentration of the active gibberellin GA4 increases dramatically prior to floral initiation but this

increase appears to be caused by a transport of GAs into the apex (maybe from the leaves) (O. Nilsson pers. com.).

### *Additional levels of regulation*

Although they are not clearly assigned to one of the main pathways, some other regulators have been shown to regulate the integrators expression or activity. Two potential chromatin-remodeling factors participate to *FT* repression. TFL2 (also called LHP1) is a heterochromatin protein counteracting *FT* induction by CO (Gaudin *et al.*, 2001, Kotake *et al.*, 2003, Takada and Goto, 2003). EBS is a nuclear protein containing a bromoadjacent homology domain repressing *FT* (Gomez-Mena *et al.*, 2001, Pineiro *et al.*, 2003). The homeobox gene *FWA* also appears to counteract FT as revealed by suppression of early-flowering phenotype of *FT* over-expression by the late-flowering *fwa* mutation (Kardailsky *et al.,* 1999; Kobayashi *et al.,* 1999). It has been shown that *FWA* is ectopically expressed due to hypomethylation in promoter repeats in semi-dominant *fwa* plants (Soppe *et al.,* 2000). However, the relevance of FWA action in a wild-type context has yet been elucidated.

## Interactions between integrators

The integrators are not only affected by the same set of pathways but they also appear to be linked to each other, thereby forming an intricate gene regulatory network (Figure 3).

### *Links between* FT *and* LFY

*LFY* expression was initially thought to be largely independent from FT. In leaf primordia of the *ft* mutant, the *LFY::GUS* reporter gene is expressed normally (*LFY* mRNA levels have not been analyzed) (Nilsson *et al.*, 1998). However, recent evidence suggests that FT might be able to induce *LFY*. *LFY* was indeed found to be downregulated in *ft* mutant plants grown in SD and shifted to LD. The difference is already obvious before the shift, suggesting that FT is involved in *LFY* progressive upregulation (Schmid *et al.*, 2003). Also, in *35S::FT* plants, *LFY* is ectopically expressed in the apical meristem and a terminal flower forms (Kardailsky *et al.*, 1999). In this later case, the *LFY* induction might be indirect. Indeed, in wild-type plants, *LFY* expression normally does not enter the shoot apical meristem because it is repressed by *TERMINAL FLOWER1* (*TFL1* ) (Ratcliffe *et al.*, 1998). Since *TFL1* encodes an FT homolog (but with opposite function), it is conceivable that an excess of FT would compete with TFL1, thereby preventing it to repress *LFY* in the apex.

### *Links between SOC1 and FT*

In an *ft* mutant, *SOC1* upregulation in the apex after a shift from SD to LD is reduced, suggesting that FT might participate in the control of *SOC1* expression (Schmid *et al.*, 2003). However this difference might just be a consequence of the delayed flowering of the *ft* mutant. Therefore, the relationship between *SOC1* and *FT* requires further investigation.

### *Links between SOC1 and LFY*

SOC1 has been propose to induce *LFY* (Jack, 2004, Lee *et al.*, 2000, Mouradov *et al.*, 2002). However there is still little data to support this attractive hypothesis. Conversion of axillary inflorescences into solitary flowers in plants overexpressing *SOC1* suggests *LFY* might be ectopically expressed in response to *SOC1* (Lee *et al.*, 2000). However, *LFY* expression has been studied neither in *soc1* mutant nor in plants overexpressing *SOC1*.

One scenario has been proposed in which *AGL24* would serve as an intermediate between *SOC1* and *LFY* (Yu *et al.*, 2002). *AGL24* encodes a MADS box protein and is expressed at the shoot apex with a sharp increase at the time of floral transition (Michaels *et al.*, 2003a, Yu *et al.*, 2002) (Figure 2). The *agl24* mutant is late flowering in both LD and SD and overexpressing *AGL24* leads to early flowering (Michaels *et al.*, 2003a, Yu *et al.*, 2002). *AGL24* is also induced by vernalization and might participate in the FLC–independent vernalization effect (Michaels *et al.*, 2003a). *AGL24* has been suggested to act downstream of *SOC1* because *AGL24* expression is decreased in *soc1* mutant (and also in *co* ) (Yu *et al.*, 2002). However, there is no consensus regarding the ability of *AGL24* constitutive expression to rescue the *soc1* mutant (Michaels *et al.*, 2003a, Yu *et al.*, 2002). In addition, AGL24 is also able to induce *SOC1* expression (Michaels *et al.*, 2003a). Understanding the interactions between *SOC1* and *AGL24* clearly requires additional work. Given that overexpression of one of the two genes has little effect when the other one is mutated (Michaels *et al.*, 2003a), one can also imagine that the two proteins works together in a MADS box complex capable of autoregulation, similar to the *AP3/PI* complex (Krizek and Meyerowitz, 1996).

AGL24 has been suggested to regulate *LFY* expression (Yu *et al.*, 2002). *LFY* expression is reduced in *agl24* mutant at the time the wild type plant flowers. In addition, *agl24* mutation do not delay flowering when *LFY* is expressed constitutively suggesting that AGL24 acts by upregulating *LFY*. It would be interesting to confirm this hypothesis by analyzing *LFY* expression before the floral transition, in order to demonstrate that reduced *LFY* expression is not just a simple consequence of *agl24* late flowering.

The linear cascade from *SOC1* to *LFY* through *AGL24* is simple and attractive. However, it requires further work to be demonstrated and will not be sufficient to explain observed phenotypes. LFY expression is not abolished in *soc1* or *agl24* mutants, *AGL24* not abolished in *soc1* mutant, indicating that other factors contribute to the upregulation of *LFY* or *AGL24*. The existence of functional redundancy between MADS-box genes might help explaining this complex situation. It is indeed quite remarkable that the three 3 MADS box genes *SOC1*, *AGL24* and *FRUITFULL* (*FUL* ) are expressed with an overlapping expression pattern (Figure 2) and all trigger early flowering when overexpressed (Ferrandiz *et al.*, 2000b, Lee *et al.*, 2000, Michaels *et al.*, 2003a, Yu *et al.*, 2002). *FUL* has received less attention probably because the *ful* single mutant is only slightly late flowering (Ferrandiz *et al.*, 2000a). Combining mutations in these three genes might reveal new roles

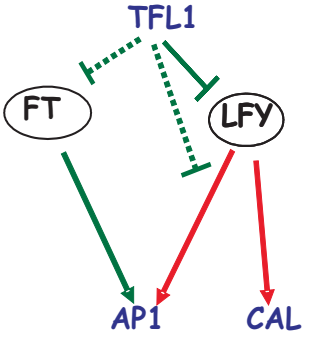


**Fig. 4. From Integrators to activation of *AP1* and *CAL*.** *Red arrows represent direct interactions, dashed arrows represent post-transcriptional regulation and plain arrows represent transcriptional regulation.*

that were not obvious from single mutant analysis. It is also possible that some of these proteins participate in heterotetrameric complexes of MADS-box genes as for floral organ identity determination (Honma and Goto, 2001). The existence of such complexes made of combination of not only activators but also of repressors (such as *SVP, FLC, FLM/MAF1 or MAF2-5* ) might provide an exquisite way to regulated flowering in response to a balance of stimulations (Hartmann *et al.*, 2000, Ratcliffe *et al.*, 2003, Scortecci *et al.*, 2003, Scortecci *et al.*, 2001).

## Downstream of the integrators

Once *FT*, *SOC1* and *LFY* have been turned on, flowering occurs. Constitutive expression of single or pairs of these integrators is already very potent at inducing 'immediate' flowering after germination. What are the molecular events initiated by the presence of floral integrators? One obvious consequence is *AP1* and *CAL* induction. However, since *AP1* overexpression is not as potent as *SOC1* or *LFY/FT* overexpression, it is likely that many more molecular events are initiated. Several recent studies at the whole genome scale identified *LFY*, *FT*, *CO* induced genes or LFY direct targets (Schmid *et al.*, 2003, Wagner *et al.*, 2004, William *et al.*, 2004). Analyzing the function of all these genes will represent of large amount of work but will certainly increase our knowledge on molecular events occurring during flowering.

### *Regulation of* AP1/CAL *by LFY*

*AP1* and *CAL* are expressed after *LFY* in the stage 1 floral meristem. In an *ap1/cal* double mutant, stage 1 and 2 flower meristems form but loose their floral fate (Bowman *et al.*, 1993). A convincing set of evidence shows that *AP1* is a direct target of LFY. a) *AP1* and *CAL* are activated in stage 1 floral meristem (figure 2), in a subpart of the domain expressing *LFY* (Kempin *et al.*, 1995, Mandel *et al.*, 1992). b) In a *lfy* mutant, *AP1* expression is strongly delayed and, in *35S::LFY* plants, *AP1* expression occurs earlier (in floral buds) but also in leaf primordia (Liljegren *et al.*, 1999, Parcy *et al.*, 1998, Ruiz-Garcia *et al.*, 1997, Weigel and Meyerowitz, 1993). c) The use of an inducible *LFY:GR* fusion demonstrated that *AP1* and *CAL* induction by LFY does not require an intermediate translational step and that the LFY protein binds to sequences present in the *AP1* promoter (Parcy *et al.*, 1998, Wagner *et al.*, 1999, William *et al.*, 2004). d) Recently, LFY binding *in vivo* to *AP1* and *CAL* regulatory sequences was demonstrated by chromosome immunoprecipitation (William *et al.*, 2004).

Several points still remain to be understood. The importance of these LFY binding sites in the *AP1* and *CAL* promoters has not yet been tested. In addition, it is known that LFY is not sufficient to activate *AP1* on its own. In yeast, LFY requires an activation domain to activate the *AP1* promoter (Parcy *et al.*, 1998). In plants also, *LFY* or *LFY-GR* constitutive expression does not constitutively induce *AP1* (Parcy *et al.*, 1998, Wagner *et al.*, 1999). LFY therefore probably needs a co-regulator to induce *AP1* and *CAL*. This coregulator could be a protein binding next to LFY (such as WUSCHEL (WUS) for *AGAMOUS* regulation (Lohmann *et al.*, 2001)) or a coactivator recruited by LFY at the *AP1* promoter.

### *Regulation of* AP1 *by FT*

As mentioned earlier, *AP1* can be induced independently of LFY. Flower like structures eventually form on a *lfy* mutant and not on a *lfy ap1* double (Huala and Sussex, 1992, Weigel *et al.*, 1992). The *AP1* expression occurring in a *lfy* mutant is absent from a *ft lfy* double mutant showing that FT is able to induce *AP1* independently of LFY (Ruiz-Garcia *et al.*, 1997). How FT induces *AP1* is not yet understood. Also, whereas *CAL,* as *AP1,* appears to be regulated by LFY, it is unclear why it cannot compensate for the loss of *AP1* in *lfy ap1* double mutant. This observation might indicate that FT is not able to induce *CAL* independently of LFY, or that CAL and AP1 meristem identity functions are not exactly equivalent.

### *A synergistic action of LFY and FT?*

Genetic data clearly illustrate that FT is able to induce *AP1* independently of LFY. However, it does not necessarily mean that FT does so during the wild-type floral transition. On the contrary, both FT and LFY appear required for the initial *AP1* induction: *AP1* induction is delayed in both the *lfy* and the *ft* single mutants suggesting that *LFY* and *FT* rather act synergistically. The FT pathway might actually represent the previously postulated LFY coregulator for *AP1* activation. A parallel with AG activation by LFY plus WUS can be drawn where LFY and WUS are thought to act synergistically in the wild type plant but still, each of them is able to induce *AG* independently of the other (Lohmann *et al.*, 2001). What is the molecular basis for LFY and FT synergistic action remains to be understood.

Interestingly, there are indications that *TFL1*, which encodes a homolog of *FT* with opposite function (Bradley *et al.*, 1996, Ratcliffe *et al.*, 1998) might also influence LFY capacity to induce *AP1*. TFL1 counteracts LFY in different ways. It prevents *LFY* expression from entering the shoot apex. In addition, when *TFL1* is constitutively expressed, LFY appears less efficient at inducing *AP1* and the *FT*-dependent *AP1* induction also does not occur (Ratcliffe *et al.*, 1999). FT and TFL1 proteins might compete antagonistically to control *AP1* upregulation by LFY. Deciphering FT and TFL1 mode of action at the molecular level is a major challenge to our understanding of floral commitment and the interplay between meristem identity genes.

## Conclusion

Tremendous progress has been realized in the last 20 years thanks to Arabidopsis genetics. After a flurry of mutant isolations, organization into a few separate pathways, cloning of the genes and analysis of their molecular function, the current picture is very different from the one two decades ago. Initially, mutations affecting "specifically" one of the pathways were the most attractive. A new class of genes has arisen that stand at the crossroads between the different pathways and integrate the influence of the environment to control the expression and activity of the floral meristem identity genes. As we pursue expression analyses, it is likely that more cross-talks between the pathways will be revealed even if they could not be guessed from genetic analyses. Progressively, the linear pathways are being integrated into a (much more realistic) gene regulatory network. Also, whereas a major focus has been initially put on gene expression at the mRNA level (probably because these experiments are the most straightforward once the gene is cloned), it is likely that analysis of protein expression and activity will reveal new links. The simple arrows

present in current models will soon become insufficient to account for the network complexity.

Also, as mentioned all along this review, the spatial aspects of the regulations have become increasingly important and analyses at the whole seedling level, as they have been performed so far, should be carefully revisited in order to draw a much more accurate picture. For instance, CO direct target genes identified using whole seedlings constitutively expressing *CO*, do not appear to be CO target in the apex during the floral transition (Samach *et al.*, 2000, Schmid *et al.*, 2003). The early distinction made between flowering time genes and meristem identity genes has been very useful in structuring the field and making it easier to follow from outside. With genes like *TFL1* or *LFY*, the frontiers have been fading and loosing part of their significance. Today, to build the gene regulatory network and understand the nature of interactions between regulators, it might be more helpful to classify proteins according to their site of action (leaf, apex, early floral meristem), even if some of them will belong to several groups. Finally, many changes occurring during flowering such as bolting or changes in phyllotaxy will have to be integrated in the global scheme.

*Acknowledgments*

*I am very grateful to O. Nilsson for inspiring discussions and precious feedback. I would like to thank T. Araki and O. Nilsson for reviewing this review, M. Blazquez, D. Weigel, C. Ferrandiz, P. Madueño, T. Araki, P. Wigge and M.C. Kim for fruitful discussions and Bab. Martin for kindly drawing plants of Figure 1.*

## References

AMASINO, R. (2004). Vernalization, competence, and the epigenetic memory of winter. *Plant Cell* 16, 2553-2559.

AN, H., ROUSSOT, C., SUAREZ-LOPEZ, P., CORBESIER, L., VINCENT, C., PINEIRO, M., HEPWORTH, S., MOURADOV, A., JUSTIN, S., TURNBULL, C., and COUPLAND, G. (2004). CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of *Arabidopsis*. *Development* 131, 3615-3626.

ARAKI, T. (2001). Transition from vegetative to reproductive phase. *Curr Opin Plant Biol* 4, 63-68.

AYRE, B.G., and TURGEON, R. (2004). Graft transmission of a floral stimulant derived from CONSTANS. *Plant Physiol* 135, 2271-2278.

BASTOW, R., and DEAN, C. (2003). Plant sciences. Deciding when to flower. Science 302, 1695-1696.

BASTOW, R., MYLNE, J.S., LISTER, C., LIPPMAN, Z., MARTIENSSEN, R.A., and DEAN, C. (2004). Vernalization requires epigenetic silencing of *FLC* by histone methylation. Nature 427, 164-167.

BERNIER, G., and PÉRILLEUX, C. (2005). A physiological overview of the genetics of flowering time control. Plant Biotechnology Journal 3, 3–16.

BERNIER, G., HAVELANGE, A., HOUSSA, C., PETITJEAN, A., and LEJEUNE, P. (1993). Physiological signals that induce flowering. Plant Cell 5, 1147-1155.

BLAZQUEZ, M.A., and WEIGEL, D. (2000). Integration of floral inductive signals in *Arabidopsis*. Nature 404, 889-892.

BLAZQUEZ, M.A., SOOWAL, L.N., LEE, I., and WEIGEL, D. (1997). *LEAFY* expression and flower initiation in *Arabidopsis*. Development 124, 3835-3844.

BLAZQUEZ, M.A., GREEN, R., NILSSON, O., SUSSMAN, M.R., and WEIGEL, D. (1998). Gibberellins promote flowering of *Arabidopsis* by activating the *LEAFY* promoter. Plant Cell 10, 791-800.

BORNER, R., KAMPMANN, G., CHANDLER, J., GLEISSNER, R., WISMAN, E., APEL, K., and MELZER, S. (2000). A MADS domain gene involved in the transition to flowering in *Arabidopsis*. Plant J 24, 591-599.

BOSS, P.K., BASTOW, R.M., MYLNE, J.S., and DEAN, C. (2004). Multiple pathways in the decision to flower: enabling, promoting, and resetting. Plant Cell 16 Suppl, S18-31.

BOWMAN, J.L., ALVAREZ, J., WEIGEL, D., MEYEROWITZ, E.M., and SMYTH, D.R. (1993). Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. Development 119, 721-743.

BRADLEY, D., CARPENTER, R., COPSEY, L., VINCENT, C., ROTHSTEIN, S., and COEN, E. (1996). Control of inflorescence architecture in *Antirrhinum*. Nature 379, 791-797.

BUSCH, M.A., BOMBLIES, K., and WEIGEL, D. (1999). Activation of a floral homeotic gene in *Arabidopsis*. Science 285, 585-587.

COLASANTI, J., and SUNDARESAN, V. (2000). 'Florigen' enters the molecular age: long-distance signals that cause plants to flower. Trends Biochem Sci 25, 236-240.

FERRANDIZ, C., LILJEGREN, S.J., and YANOFSKY, M.F. (2000a). Negative regulation of the *SHATTERPROOF* genes by *FRUITFULL* during *Arabidopsis* fruit development. Science 289, 436-438.

FERRANDIZ, C., GU, Q., MARTIENSSEN, R., and YANOFSKY, M.F. (2000b). Redundant regulation of meristem identity and plant architecture by *FRUITFULL*, *APETALA1* and *CAULIFLOWER*. Development 127, 725-734.

GAUDIN, V., LIBAULT, M., POUTEAU, S., JUUL, T., ZHAO, G., LEFEBVRE, D., and GRANDJEAN, O. (2001). Mutations in *LIKE HETEROCHROMATIN PROTEIN 1* affect flowering time and plant architecture in *Arabidopsis*. Development 128, 4847-4858.

GOCAL, G.F., SHELDON, C.C., GUBLER, F., MORITZ, T., BAGNALL, D.J., MACMILLAN, C.P., LI, S.F., PARISH, R.W., DENNIS, E.S., WEIGEL, D., and KING, R.W. (2001). *GAMYB*-like genes, flowering, and gibberellin signaling in *Arabidopsis*. Plant Physiol 127, 1682-1693.

GOMEZ-MENA, C., PINEIRO, M., FRANCO-ZORRILLA, J.M., SALINAS, J., COUPLAND, G., and MARTINEZ-ZAPATER, J.M. (2001). early bolting in short days: an *Arabidopsis* mutation that causes early flowering and partially suppresses the floral phenotype of *leafy*. Plant Cell 13, 1011-1024.

HARTMANN, U., HOHMANN, S., NETTESHEIM, K., WISMAN, E., SAEDLER, H., and HUIJSER, P. (2000). Molecular cloning of *SVP*: a negative regulator of the floral transition in *Arabidopsis*. Plant J 21, 351-360.

HE, Y., MICHAELS, S.D., and AMASINO, R.M. (2003). Regulation of flowering time by histone acetylation in *Arabidopsis*. Science 302, 1751-1754.

HEMPEL, F.D., and FELDMAN, L.J. (1993). Bi-directional inflorescence development in *Arabidopsis thaliana*: Acropetal initiation of flowers and basipetal initiation of paraclades. Planta 192, 276-286.

HENDERSON, I.R., and DEAN, C. (2004). Control of *Arabidopsis* flowering: the chill before the bloom. Development 131, 3829-3838.

HEPWORTH, S.R., VALVERDE, F., RAVENSCROFT, D., MOURADOV, A., and COUPLAND, G. (2002). Antagonistic regulation of flowering-time gene *SOC1* by *CONSTANS* and *FLC* via separate promoter motifs. Embo J 21, 4327-4337.

HONMA, T., and GOTO, K. (2001). Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature 409, 525-529.

HUALA, E., and SUSSEX, I.M. (1992). *LEAFY* interacts with Floral Homeotic genes to regulate *Arabidopsis* floral development. Plant Cell 4, 901-903.

JACK, T. (2004). Molecular and genetic mechanisms of floral control. Plant Cell 16 Suppl, S1-17.

JOHANSON, U., WEST, J., LISTER, C., MICHAELS, S., AMASINO, R., and DEAN, C. (2000). Molecular analysis of *FRIGIDA*, a major determinant of natural variation in *Arabidopsis* flowering time. Science 290, 344-347.

KARDAILSKY, I., SHUKLA, V.K., AHN, J.H., DAGENAIS, N., CHRISTENSEN, S.K., NGUYEN, J.T., CHORY, J., HARRISON, M.J., and WEIGEL, D. (1999). Activation tagging of the floral inducer *FT*. Science 286, 1962-1965.

KEMPIN, S.A., SAVIDGE, B., and YANOFSKY, M.F. (1995). Molecular basis of the *cauliflower* phenotype in *Arabidopsis*. Science 267, 522-525.

KIEFFER, M., and DAVIES, B. (2001). Developmental programmes in floral organ formation. Semin Cell Dev Biol 12, 373-380.

KOBAYASHI, Y., KAYA, H., GOTO, K., IWABUCHI, M., and ARAKI, T. (1999). A pair of related genes with antagonistic roles in mediating flowering signals. Science 286, 1960-1962.

KOORNNEEF, M., HANHART, C.J., and VAN DER VEEN, J.H. (1991). A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol. Gen. Genet. 229, 57-66.

KOTAKE, T., TAKADA, S., NAKAHIGASHI, K., OHTO, M., and GOTO, K. (2003). *Arabidopsis TERMINAL FLOWER 2* gene encodes a HETEROCHROMATIN PROTEIN 1 homolog and represses both *FLOWERING LOCUS T* to regulate flowering time and several floral homeotic genes. Plant Cell Physiol 44, 555-564.

KRIZEK, B.A., and MEYEROWITZ, E.M. (1996). The *Arabidopsis* homeotic genes *APETALA3* and *PISTILLATA* are sufficient to provide the B class organ identity function. Development 122, 11-22.

LEE, H., SUH, S.S., PARK, E., CHO, E., AHN, J.H., KIM, S.G., LEE, J.S., KWON, Y.M., and LEE, I. (2000). The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. Genes Dev 14, 2366-2376.

LILJEGREN, S.J., GUSTAFSON-BROWN, C., PINYOPICH, A., DITTA, G.S., and YANOFSKY, M.F. (1999). Interactions among *APETALA1*, *LEAFY*, and *TERMINAL FLOWER1* specify meristem fate. Plant Cell 11, 1007-1018.

LOHMANN, J.U., and WEIGEL, D. (2002). Building beauty: the genetic control of floral patterning. Dev Cell 2, 135-142.

LOHMANN, J.U., HONG, R.L., HOBE, M., BUSCH, M.A., PARCY, F., SIMON, R., and WEIGEL, D. (2001). A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. Cell 105, 793-803.

LONG, J., and BARTON, M.K. (2000). Initiation of axillary and floral meristems in *Arabidopsis*. Dev Biol 218, 341-353.

MANDEL, M.A., GUSTAFSON-BROWN, C., SAVIDGE, B., and YANOFSKY, M.F. (1992). Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. Nature 360, 273-277.

MARTINEZ-ZAPATER, J.M., COUPLAND, G., DEAN, C., and KOORNNEEF, M. (1994). The transition to flowering in *Arabidopsis*. In *Arabidopsis*, E.M. Meyerowitz and C.R. Somerville, eds (New-York: Cold Spring Harbor Laboratory Press), pp. 403-434.

MICHAELS, S.D., and AMASINO, R.M. (1999). *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11, 949-956.

MICHAELS, S.D., HE, Y., SCORTECCI, K.C., and AMASINO, R.M. (2003a). Attenuation of *FLOWERING LOCUS C* activity as a mechanism for the evolution of summer-annual flowering behavior in *Arabidopsis*. Proc Natl Acad Sci U S A 100, 10102-10107.

MICHAELS, S.D., HIMELBLAU, E., KIM, S.Y., SCHOMBURG, F.M., and AMASINO, R.M. (2005). Integration of flowering signals in winter-annual *Arabidopsis*. Plant Physiol 137, 149-156.

MICHAELS, S.D., DITTA, G., GUSTAFSON-BROWN, C., PELAZ, S., YANOFSKY, M., and AMASINO, R.M. (2003b). AGL24 acts as a promoter of flowering in *Arabidopsis* and is positively regulated by vernalization. Plant J 33, 867-874.

MOON, J., LEE, H., KIM, M., and LEE, I. (2005). Analysis of flowering pathway integrators in *Arabidopsis*. Plant Cell Physiol.

MOON, J., SUH, S.S., LEE, H., CHOI, K.R., HONG, C.B., PAEK, N.C., KIM, S.G., and LEE, I. (2003). The *SOC1* MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. Plant J 35, 613-623.

MOURADOV, A., CREMER, F., and COUPLAND, G. (2002). Control of flowering time: interacting pathways as a basis for diversity. Plant Cell 14, S111-130.

NILSSON, O., LEE, I., BLAZQUEZ, M.A., and WEIGEL, D. (1998). Flowering-time genes modulate the response to *LEAFY* activity. Genetics 150, 403-410.

NOH, Y.S., and AMASINO, R.M. (2003). *PIE1*, an ISWI family gene, is required for *FLC* activation and floral repression in *Arabidopsis*. Plant Cell 15, 1671-1682.

NOH, Y.S., BIZZELL, C.M., NOH, B., SCHOMBURG, F.M., and AMASINO, R.M. (2004). *EARLY FLOWERING 5* acts as a floral repressor in *Arabidopsis*. Plant J 38, 664-672.

ONOUCHI, H., IGENO, M.I., PERILLEUX, C., GRAVES, K., and COUPLAND, G. (2000). Mutagenesis of plants overexpressing *CONSTANS* demonstrates novel interactions among *Arabidopsis* flowering-time genes. Plant Cell 12, 885-900.

PARCY, F., BOMBLIES, K., and WEIGEL, D. (2002). Interaction of *LEAFY*, *AGAMOUS* and *TERMINAL FLOWER1* in maintaining floral meristem identity in *Arabidopsis*. Development 129, 2519-2527.

PARCY, F., NILSSON, O., BUSH, M.A., LEE, I., and WEIGEL, D. (1998). A genetic framework for floral patterning. Nature 395, 561-566.

PINEIRO, M., GOMEZ-MENA, C., SCHAFFER, R., MARTINEZ-ZAPATER, J.M., and COUPLAND, G. (2003). EARLY BOLTING IN SHORT DAYS is related to chromatin remodeling factors and regulates flowering in *Arabidopsis* by repressing FT. Plant Cell 15, 1552-1562.

PUTTERILL, J., ROBSON, F., LEE, K., SIMON, R., and 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.

RATCLIFFE, O.J., BRADLEY, D.J., and COEN, E.S. (1999). Separation of shoot and floral identity in *Arabidopsis*. Development 126, 1109-1120.

RATCLIFFE, O.J., KUMIMOTO, R.W., WONG, B.J., and RIECHMANN, J.L. (2003). Analysis of the *Arabidopsis MADS AFFECTING FLOWERING* gene family: *MAF2* prevents vernalization by short periods of cold. Plant Cell 15, 1159-1169.

RATCLIFFE, O.J., AMAYA, I., VINCENT, C.A., ROTHSTEIN, S., CARPENTER, R., COEN, E.S., and BRADLEY, D.J. (1998). A common mechanism controls the life cycle and architecture of plants. Development 125, 1609-1615.

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

SAMACH, A., ONOUCHI, H., GOLD, S.E., DITTA, G.S., SCHWARZ-SOMMER, Z., YANOFSKY, M.F., and COUPLAND, G. (2000). Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. Science 288, 1613-1616.

SCHMID, M., UHLENHAUT, N.H., GODARD, F., DEMAR, M., BRESSAN, R., WEIGEL, D., and LOHMANN, J.U. (2003). Dissection of floral induction pathways using global expression analysis. Development 130, 6001-6012.

SCORTECCI, K., MICHAELS, S.D., and AMASINO, R.M. (2003). Genetic interactions between *FLM* and other flowering-time genes in *Arabidopsis thaliana*. Plant Mol Biol 52, 915-922.

SCORTECCI, K.C., MICHAELS, S.D., and AMASINO, R.M. (2001). Identification of a MADS-box gene, *FLOWERING LOCUS M*, that represses flowering. Plant J 26, 229-236.

SESSIONS, A., YANOFSKY, M.F., and WEIGEL, D. (2000). Cell-cell signaling and movement by the floral transcription factors LEAFY and APETALA1. Science 289, 779-782.

SIMON, R., IGEÑO, M.I., and COUPLAND, G. (1997). Activation of floral meristem identity genes in *Arabidopsis*. Nature 382, 59-62.

SIMPSON, G.G., and DEAN, C. (2002). *Arabidopsis*, the Rosetta stone of flowering time? Science 296, 285-289.

SIMPSON, G.G., GENDALL, A.R., and DEAN, C. (1999). When to switch to flowering. Annu Rev Cell Dev Biol 15, 519-550.

SMYTH, D.R., BOWMAN, J.L., and MEYEROWITZ, E.M. (1990). Early flower development in *Arabidopsis*. Plant Cell 2, 755-767.

SUAREZ-LOPEZ, P., WHEATLEY, K., ROBSON, F., ONOUCHI, H., VALVERDE, F., and COUPLAND, G. (2001). CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*. Nature 410, 1116-1120.

SUH, S.S., CHOI, K.R., and LEE, I. (2003). Revisiting phase transition during flowering in *Arabidopsis*. Plant Cell Physiol 44, 836-843.

SUNG, S., and AMASINO, R.M. (2004a). Vernalization and epigenetics: how plants remember winter. Curr Opin Plant Biol 7, 4-10.

SUNG, S., and AMASINO, R.M. (2004b). Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. Nature 427, 159-164.

TAKADA, S., and 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. Plant Cell 15, 2856-2865.

VALVERDE, F., MOURADOV, A., SOPPE, W., RAVENSCROFT, D., SAMACH, A., and COUPLAND, G. (2004). Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science 303, 1003-1006.

WAGNER, D., and MEYEROWITZ, E.M. (2002). SPLAYED, a novel SWI/SNF ATPase homolog, controls reproductive development in *Arabidopsis*. Curr Biol 12, 85-94.

WAGNER, D., SABLOWSKI, R.W., and MEYEROWITZ, E.M. (1999). Transcriptional activation of *APETALA1* by *LEAFY*. Science 285, 582-584.

WAGNER, D., WELLMER, F., DILKS, K., WILLIAM, D., SMITH, M.R., KUMAR, P.P., RIECHMANN, J.L., GREENLAND, A.J., and MEYEROWITZ, E.M. (2004). Floral induction in tissue culture: a system for the analysis of *LEAFY*-dependent gene regulation. Plant J 39, 273-282.

WEIGEL, D., and MEYEROWITZ, E.M. (1993). *LEAFY* controls meristem identity in *Arabidopsis*. In Cellular Communications in Plants, R. Amasino, ed (New York: Plenum Press), pp. 115-122.

WEIGEL, D., and MEYEROWITZ, E.M. (1994). Morphology and development of *leafy* mutants. In *Arabidopsis*: An Atlas of Morphology and Development, J.L. Bowman, ed (New York: Springer), pp. 206-211.

WEIGEL, D., and NILSSON, O. (1995). A developmental switch sufficient for flower initiation in diverse plants. Nature 377, 495-500.

WEIGEL, D., ALVAREZ, J., SMYTH, D.R., YANOFSKY, M.F., and MEYEROWITZ, E.M. (1992). *LEAFY* controls floral meristem identity in *Arabidopsis*. Cell 69, 843-859.

WILLIAM, D.A., SU, Y., SMITH, M.R., LU, M., BALDWIN, D.A., and WAGNER, D. (2004). Genomic identification of direct target genes of LEAFY. Proc Natl Acad Sci U S A 101, 1775-1780.

WILSON, R.N., HECKMAN, J.W., and SOMERVILLE, C.R. (1992). Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. Plant Physiol. 100, 403-408.

WU, X., DINNENY, J.R., CRAWFORD, K.M., RHEE, Y., CITOVSKY, V., ZAMBRYSKI, P.C., and WEIGEL, D. (2003). Modes of intercellular transcription factor movement in the *Arabidopsis* apex. Development 130, 3735-3745.

YU, H., XU, Y., TAN, E.L., and KUMAR, P.P. (2002). *AGAMOUS-LIKE 24*, a dosage-dependent mediator of the flowering signals. Proc Natl Acad Sci U S A 99, 16336-16341.

YU, H., ITO, T., WELLMER, F., and MEYEROWITZ, E.M. (2004). Repression of *AGAMOUS-LIKE 24* is a crucial step in promoting flower development. Nat Genet 36, 157-161.

ZEEVAART, J.A.D. (1976). Physiology of flower formation. Ann.Rev. Plant. Physiol. 27, 321-348.