Control of flowering time

Yaron Y Levy* and Caroline Dean†

The multiple promotive and repressive pathways controlling flowering have been further defined by analysis of genetic interactions and the activation of floral meristem identity genes. Cloning of additional genes in these pathways has uncovered some of the molecular processes that control the timing of the transition to reproductive development.

Addresses
Department of Molecular Genetics, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK;
*e-mail: levy@bbsrc.ac.uk
†e-mail: caroline.dean@bbsrc.ac.uk

Current Opinion in Plant Biology 1998, 1:49–54

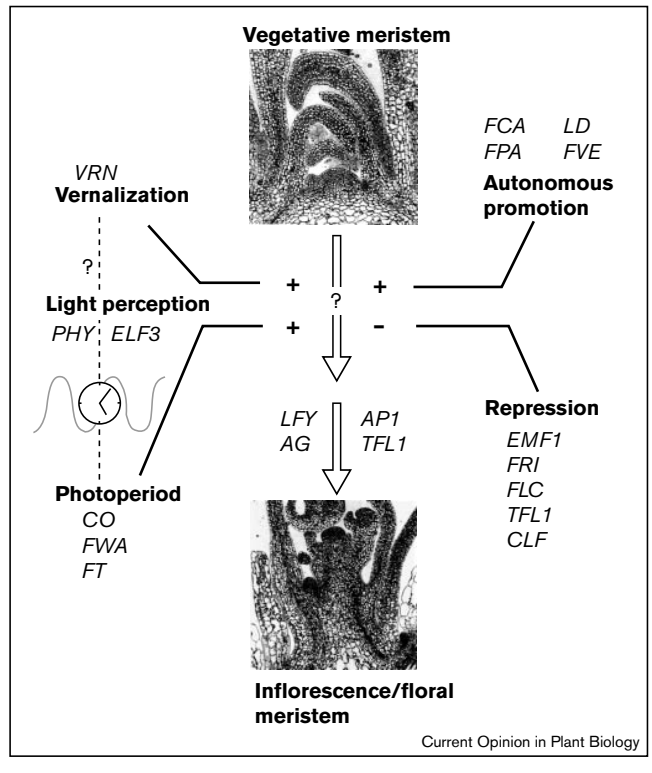
http://biomednet.com/elecref/1369526600100049

Abbreviations
GA gibberellin
PHY phytochrome

Introduction

To reproduce under favourable conditions, plants moderate their intrinsic developmental timing with cues from the environment, particularly day length, light quality, and temperature. Physiological studies have led to a general 'multifactorial model' [1] which attempts to account for the diverse flowering responses observed in a variety of species. In this model, growth regulators and assimilates act as floral promoters and inhibitors which are required in appropriate concentrations and at particular times before flowering is triggered. The genetics of flowering time support this model [2••,3,4] (Figure 1). Multiple genetic pathways have been identified, some of which promote flowering and some of which repress it. Some genes act independently of growth conditions, while others mediate responses to environmental cues. In this review, we focus on recent advances in our understanding of the control of flowering time, drawing mainly from work with *Arabidopsis* as a model system.

Figure 1



Physiological pathways and genes controlling flowering in *Arabidopsis*. Physiological studies have identified different pathways that either promote (+) or repress (−) the transition of the apical meristem from vegetative to inflorescence/floral development. Only genes specifically mentioned in the text are included in this figure. The circadian clock (left side of figure) is implicated in the measurement of photoperiod via the perception of light. *Arabidopsis* strains in which flowering is promoted by vernalization also show strong acceleration of flowering by far-red-enriched light, so vernalization and perception of light quality appear to be closely related processes. The inputs from the different pathways are somehow integrated (symbolised by a question mark) and eventually lead to activation of inflorescence/floral meristem identity genes. A major future goal is to analyse the interactions of the different genes to define these physiological processes in terms of genetic pathways.

Just do it: autonomous promotion

The autonomous promotion pathway (Figure 1) is considered to promote the transition from vegetative growth to flowering, independently of environmental cues. Cloning and analysis of the *FCA* gene, a component of this pathway, demonstrated a role for post-transcriptional regulation in this pathway [5••]. The FCA protein is an RNA-binding protein with a high degree of similarity, within the RNA-recognition motifs, to *Drosophila* proteins SX-1 and ELAV. These proteins function in fly development to alternatively splice transcripts in the sex-determination and neuronal differentiation pathways respectively. The *FCA* transcript itself is alternatively spliced and increasing the levels of certain *FCA* transcripts resulted in earlier flowering. This result suggests that FCA is part of a post-transcriptional regulatory cascade in which alternative RNA splicing is an important point of control.

The rather general expression of *FCA* throughout the plant was similar to that observed for another gene of the autonomous promotion pathway, *LD* [6], and is consistent with genes in this pathway functioning throughout development [7]. Furner *et al.* [8] used X-rays to generate plants with sectors of *fca* tissue in an otherwise wild-type background. Analysis of *fca* sectors in the two inner layers (L2 and L3), which were marked by loss

of a gene near *FCA* involved in pigmentation, showed that FCA is not required in the entire apical meristem in order to produce a phenotypically normal plant [8]. These results imply that FCA or downstream signals produced in the L1 (epidermal) layer or in adjacent wild-type L2/L3 tissue can diffuse within the plant and rescue the phenotype of the *fca* sectors. Translocated signals which promote flowering have been demonstrated in the elegant genetic analysis of flowering time in pea [3]. The phenotype of the pea mutant *gigas*, which is deficient in a graft-transmissible floral stimulus and is more responsive to vernalization, has led to speculation about whether *GIGAS* is a pea orthologue of *FCA* [9••].

Seeing the light: photoperiodic induction

Many plants monitor day length (figure 1) as a cue for flowering at the correct time of year. The promotion of flowering by photoperiod has received considerable attention over the years with clear evidence that promotive and repressive signals, made in the leaves, are translocated in the phloem exudate to the apex [1]. While there have been considerable efforts to identify transcripts and substances induced in leaves that might form the basis of this floral signal, specific candidates remain elusive [10–12]. The recent development of a method to induce synchronous flowering in *Arabidopsis* in response to one long day photoperiod [13] will facilitate the important integration of genetics and physiology studies when similar experiments are done with *Arabidopsis* mutants. An exciting recent development has been the demonstration that there is a connection between the endogenous circadian clock and the control of flowering time, probably via the photoperiodic promotion pathway (Figure 1). Mutations at the *ELF3* locus result in an elongated hypocotyl (especially under blue light) and early flowering which is insensitive to photoperiod [14]. *elf3* shows no circadian rhythm in continuous light, thus showing a connection between the circadian clock and the control of flowering time [15•] and suggesting a role for *ELF3* in linking light perception to circadian rhythms.

There has also been a focus on regulatory events occurring at the shoot apex as it switches from vegetative to reproductive development. Experiments with cultured apices of *Lolium* demonstrated that photoperiodic induction resulted from two signals acting at the apex [16]. The first signal, of an unknown nature, switched the developmental fate of the shoot meristem cells from commitment to produce leaves to commitment to produce flowers, and then the second signal, gibberellin (GA), triggered expression of this florally determined state. GAs are also likely to be responsible for the acceleration of primordium initiation at the apex, an early manifestation of induction by long day photoperiods [17]. Experiments in *Sinapis* aimed at identifying transcripts expressed in the apex in response to long day photoperiods demonstrated the induction of two MADS box transcription factor genes [18] and a gene, *FPF1*, which is possibly involved in GA signaling [19]. GAs are clearly involved in multiple processes related to flowering, and the interaction of GAs and phytochrome-mediated signaling pathways is complex. Analysis of mutants deficient in both phytochrome and GA responses has shown that a fully functional GA system is necessary for the full expression of at least one manifestation of phytochrome deficiency, an elongated hypocotyl [20]. Increased responsiveness of *phyB* mutants to exogenous GAs [21] and the interesting phenomenon of floral meristem reversion [22] also suggest an interaction between phytochrome and GA signaling.

Vernalization: promotion by cold temperature

A long cold temperature treatment (i.e., a winter season) induces or accelerates flowering in many species. This phenomenon, known as vernalization, has a number of unusual features that suggest an epigenetic mechanism as its basis [23]. Burn *et al.* [24] proposed that vernalization causes general DNA demethylation which allows expression of kaurenoic acid hydroxylase, an important enzyme in GA biosynthesis. This hypothesis was tested by transforming *Arabidopsis* plants with a construct expressing an antisense transcript of a methyltransferase gene (*MET1*), resulting in plants with substantially reduced levels of cytosine methylation [25]. Many developmental abnormalities were seen in these plants, but consistent with the above hypothesis, the antisense methyltransferase plants flowered earlier than the wild-type [26]. Two other studies have also addressed the role of methylation in flowering. Ronemus *et al.* [27], using a similar *MET1*-antisense construct, and Kakutani *et al.* [28], working with the *ddm1* mutant, which has decreased DNA methylation but unaltered methyltransferase activity, noted late flowering as a frequently appearing phenotype in their plant lines. These data imply that methylation has a role in establishing or maintaining different developmental states of the meristem; Ronemus *et al.* [27] speculate that there is a gradient of increasing methylation during development, acting to change meristem competency and determinacy. It would be interesting, therefore, to establish whether this gradual increase in methylation during development is related to the changes in floral repressor concentration proposed by the 'controller of phase switch' hypothesis [29]. The level of repressor activity is proposed to decrease over time due to an internal (developmental) program, as well as being modulated by external (environmental) signals. Switches in phase (e.g., from inflorescesce to floral development) are proposed to occur when repressor activity drops below the critical level for maintaining the current phase.

Another approach to understanding the molecular basis of vernalization has been to identify and analyse mutants that are impaired in the vernalization response [30•]. Thus, the *vrn1* and *vrn2* mutants were isolated by mutagenising the late-flowering vernalization-responsive *fca-1* mutant followed by selection for individuals exhibiting a reduced vernalization response. The *vrn1* mutation

reduced the vernalization response of other late-flowering vernalization-responsive mutants and of Landsberg *erecta*, an early flowering genotype of *Arabidopsis*, under noninductive photoperiods [30•]. Thus *VRN1* appears to be a component of the vernalization promotion pathway (Figure 1). *Arabidopsis* mutants and ecotypes that show a strong response to vernalization also show an acceleration of flowering in response to receiving a low ratio of red to far-red light (for example [31,32]) thus indicating a response mediated via phytochrome. A recent study showed that mutants deficient in both phytochrome A and B still respond to far-red light by flowering early, implicating other phytochromes in this response [33]. Interestingly, both *vrn1* and *vrn2* display additional photomorphogenic phenotypes (our unpublished data), further linking light quality perception with vernalization. Analysis of the *VRN* genes should identify the molecular processes important in vernalization and may clarify the connection between vernalization and light quality perception.

Not so fast: repression of flowering

In *Arabidopsis*, the identification of recessive mutations that cause early flowering [34], in some cases with no vegetative growth at all (e.g. *embryonic flowering 1*, *emf1*), suggests that flowering is normally actively repressed beginning from embryonic development. Physiological and genetic experiments with tobacco [35] and pea [3] have shown that the roots and leaves respectively, of these plants produce a substance which represses flowering. The synthesis or transport of the inhibitor produced in pea leaves is reduced by a PHYA-mediated signaling pathway [36]. A major gene that represses flowering in *Arabidopsis* is *FRIGIDA* (*FRI*), with dominant alleles causing late flowering and conferring a winter growth habit [37] (Figure 1). Recently, Sanda *et al.* [38•] extended the known range of ecotypes in which *FRI* has been shown to be the major determinant of flowering time in natural populations. Repression of flowering by *FRI* requires dominant alleles at a second locus, *FLC* [39]. Synergistic interactions were found between *FLC* and mutants impaired in the autonomous promotion of flowering (*fca*, *fpa*, and *fve*) suggesting that *FLC* acts antagonistically to *FCA*, *FPA* and *FVE* function [40•]. In other words, these results support the notion that *FLC* contributes to repression of flowering which is antagonistic to the autonomous promotion pathway. Orthologues of *FRI* and *FLC* are likely to be important in the control of flowering in other species; for example, the two major quantitative trait loci conferring vernalization requirement in *Brassica* species cosegregate with markers linked to *FRI* and *FLC* [41•].

Taking the next step: where timing and meristem identity meet

At some point in time, the balance or levels of promotive and repressive factors is such that flowering is triggered. Classically, the vegetative meristem is thought to become competent to respond to inductive signals and then at a certain point to be 'evoked' into a florally determined state. Thus evocation is defined as 'the events that occur in the apex that commit it to flower' [42] and may be defined at the molecular level by the expression of genes regulating meristem identity, such as *LEAFY* (*LFY*), *TERMINAL FLOWER 1* (*TFL1*), and *APETALA 1* (*AP1*) [43] (Figure 1). Recently, the temporal sequence of *LFY* and *AP1* expression during the induction of flowering has been established in relation to 'determination' [44], that is, the point at which the developmental fate of the meristem cells is switched. An increase in *LFY* expression preceded determination, while *AP1* expression was always first observed after determination. Blazquez *et al.* [45] have shown that *LFY* expression increased rapidly and dramatically when plants were shifted from non-inductive to inductive photoperiods, suggesting that the *LFY* promoter is a target of photoperiodic promotion. The *CONSTANS* (*CO*) gene (Figure 1) promotes flowering in response to long days [46]. A system in which wild-type CO protein could be inducibly activated in *co* mutant plants allowed the role of CO in the expression of meristem identity genes to be analysed [47••]. CO was sufficient to induce flowering and to initiate transcription of *LFY* and *TFL1* in *co* plants as rapidly as when these genes are induced by long day photoperiods in wild-type plants. *AP1* transcription, however, was induced more slowly by CO compared to long day photoperiods in wild-type plants. Simon *et al.* [47••] conclude that CO acts in a pathway that is sufficient to activate *LFY* and *TFL1* transcription and that rapid activation of *AP1* requires an additional pathway. Proteins from *Arabidopsis* and *Antirrhinum* (snapdragon) that recognise and bind to regions of the *AP1/SQUAMOSA* promoter have recently been identified [48,49] and will aid understanding of how *AP1* expression is connected to the control of flowering time. *FWA* and *FT*, two genes defined by mutations causing late flowering (Figure 1), also appear necessary for the function of some of the genes affecting meristem identity [50] as *lfy fwa* or *lfy ft* double mutants show a severe inflorescence phenotype, stronger than that in *lfy ap1* double mutants, where no flower-like structures were produced. Whether any of the genes that control flowering time directly regulate any of the meristem identity genes, such as *LFY*, remains to be seen.

There are now several examples of mutated flowering time genes which cause the plants to exhibit altered inflorescence or floral morphology (e.g. [14,51]), and mutated meristem identity genes which cause altered flowering time. The *terminal flower 1* (*tfl1*) mutant shows accelerated transitions from vegetative to inflorescence formation and from secondary inflorescence to flower production [52]. The inflorescence also becomes determinate, showing that wild-type *TFL1* function is involved in the suppression of flower formation at the apex thus resulting in the normally indeterminate inflorescence. The *Arabidopsis TFL1* gene has been cloned by virtue of

its homology to the *Antirrhinum* orthologue *CENTRORADIALIS* [53••] and by T-DNA insertional mutagenesis [54]. *TFL1* encodes a protein with limited similarity to animal phosphatidylethanolamine-binding proteins which can associate with membrane protein complexes. An example of an early flowering mutant with altered meristem identity gene expression is *curly leaf* (*clf*). The *clf* mutant was shown to express the *AGAMOUS* (*AG*) gene ectopically [55••]. The *AG* gene product is normally required to direct stamen and carpel development in the flower. Thus *CLF* function represses *AG* transcription in leaves, inflorescence stems and flowers. The ectopic expression of *AG* in the *clf* mutant results in early flowering and curling of the leaves. A transposon-tagged allele of *clf* enabled the gene to be cloned; it encodes a protein with extensive homology to a *Drosophila* Polycomb-group (Pc-G) gene, required for repression of homeotic gene activity in fly development [55••].

Conclusions

The increasing number of flowering time genes that have been cloned will provide a basis for teasing apart the regulatory pathways that control the transition from vegetative growth to flowering. A major challenge is to define the epistatic relationships among genes involved in flowering time. A recent analysis of epistasis among ten late flowering *Arabidopsis* mutants [56] has revealed that the interaction of these genes is more complicated than originally thought. Furthermore, newly identified floral promoters (e.g. [51,57]) and repressors will need to be incorporated into the genetic model of the control of flowering time. Clearly much needs to be done, but the continued integration of studies involving physiology and molecular genetics will provide exciting discoveries into how plants balance the internal and external signals which control the transition to reproductive development.

Acknowledgements

We would like to thank the members of the Dean laboratory for discussions and critical comments on the manuscript. Our work on genes that control flowering time is supported by BBSRC CSG to the John Innes Centre, BBSRC grants PG208/0606 and AT208/578, and European community grant BIO2 CT93 0400.

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