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Induction of flowering by seasonal changes in photoperiod

Iain Searle and George Coupland*

Max Planck Institute for Plant Breeding, Carl von Linne Weg, Cologne, Germany

In many plants, major developmental transitions such as the initiation of flowering are synchronized to the changing seasons. Day length provides one of the environmental cues used to achieve this. We describe the molecular mechanisms that measure day length and control flowering in *Arabidopsis*. Also, we compare these mechanisms with those that control flowering time in rice. This comparison suggests that components of the *Arabidopsis* regulatory network are conserved in other species, but that their regulation can be altered to generate different phenotypic responses.

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Introduction

The life cycle of many plants is synchronized to the changing seasons. This pattern of behaviour ensures that developmental transitions, such as the onset of flowering, occur under the most appropriate environmental conditions and in many locations is an essential aspect of the sessile growth habit of plants. Fluctuations in day length (or photoperiod) and temperature provide the information used to synchronize these developmental decisions to the seasons. The mechanism underlying photoperiodic responses has been of interest since they were first described in detail in the 1920s (Garner and Allard, 1920). A conceptual breakthrough was the realization that a circadian clock, an endogenous timing mechanism with a cycle time or period length of approximately 24 h, is the time-keeping mechanism required to measure day length (Bünning, 1936; Thomas and Vince-Prue, 1997). This was later refined as a coincidence model in which exposure of a plant to light at a particular phase of a circadian rhythm would trigger or repress a developmental transition (Pittendrigh and Minis, 1964). Such a system would consist of two parts: a circadian rhythm in a component that regulates the developmental response and whose activity is controlled by exposure of the plant to light, and a light signalling pathway that activates or represses the activity of this component. Genetic analysis of the control of flowering has identified genes that confer a photoperiodic response on *Arabidopsis* and suggested a molecular basis for the coincidence between circadian rhythms and light (Hayama and Coupland, 2003; Yanovsky and Kay, 2003). In this review, we describe the mechanisms that underlie the response to day length in *Arabidopsis*, and how these are modified in other plant species.

*Corresponding author. Department of Plant Developmental Biology, Max Planck Institute for Plant Breeding, Carl von Linne Weg, 10, D-50829 Cologne, Germany. Tel.: +49 221 5062 205; Fax: +49 221 5062 207; E-mail: coupland@mpiz-koeln.mpg.de

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A regulatory pathway that induces flowering of *Arabidopsis* in response to photoperiod

Arabidopsis shows a strong photoperiod response in the onset of flowering, and most strains (or accessions) flower in spring or early summer as the days become longer. In laboratory conditions, flowering occurs much earlier under long days of 16 h light than under short days of 10 h light. Mutations that disrupt these responses were isolated by identifying mutants with a reduced response to day length (Redei, 1962; Koornneef *et al*, 1991). These mutants fell into two classes, those that flower later than wild-type plants under long days but are unaffected under short days or, alternatively, early-flowering mutants under short days.

Some of the mutations that cause early flowering under short days also cause a general disruption of circadian rhythms. In *Arabidopsis*, behaviours such as leaf movements or the elongation of cells in the hypocotyl (Dowson-Day and Millar, 1999) as well as the expression of around 6% of genes (Harmer *et al*, 2000; Schaffer *et al*, 2001) are under circadian clock control. The mutations that reduce day-length responses by causing early flowering under short days also cause a general disruption of these circadian rhythms. These include mutations in the *EARLY FLOWERING3* (*ELF3*), *TIMING OF CHLOROPHYLL A/B BINDING PROTEIN1* (*TOC1*), *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) genes (Table I). The expression of these genes is also regulated by the circadian clock, so that their mRNAs only accumulate in the morning (*LHY* and *CCA1*) or the evening (*TOC1* and *ELF3*).

LHY, *CCA1* and *TOC1* may be part of the central mechanism that generates circadian rhythms in plants. CCA1 and LHY are similar in sequence and expression pattern (Schaffer *et al*, 1998; Wang and Tobin, 1998), and are genetically partially redundant (Alabadi *et al*, 2002; Mizoguchi *et al*, 2002). In the *lhy cca1* double mutant or the *toc1* single mutant, circadian rhythms cycle faster and the plants flower

Table I Proteins involved in the response to day length in *Arabidopsis*

Proteins	Putative biochemical function	References
EARLY FLOWERING3 (ELF3)	Nuclear protein proposed to act as a transcriptional activator	McWatters *et al* (2000), Hicks *et al* (2001) and Liu *et al* (2001b)
TIMING OF CHLOROPHYLL A/B BINDING PROTEIN1 (TOC1)	N-terminus is similar to the receiver domain of bacterial response regulators; C-terminus is the plant-specific CCT domain	Strayer *et al* (2000)
LATE ELONGATED HYPOCOTYL (LHY)	Myb domain DNA binding	Schaffer *et al* (1998) and Wang and Tobin (1998)
CIRCADIAN CLOCK ASSOCIATED1 (CCA1)	Myb domain DNA binding	Schaffer *et al* (1998) and Wang and Tobin (1998)
CONSTANS (CO)	Nuclear protein containing two B-box zinc fingers; C-terminus CCT domain	Putterill *et al* (1995) and Robson *et al* (2001)
GIGANTEA (GI)	Nuclear protein of unknown function	Fowler *et al* (1999) and Park *et al* (1999)
FLOWERING LOCUS T (FT)	Homology to RAF kinase inhibitor	Kardailsky *et al* (1999) and Kobayashi *et al* (1999)
SUPPRESSOR OF OVEREXPRESSION OF CONSTANS (SOC1)	MADS box transcription factor	Borner *et al* (2000), Lee *et al* (2000) and Samach *et al* (2000)
CRYPTOCHROME 2 (CRY2)	Blue light photoreceptor involved in the post-transcriptional regulation of CO	El-Assal *et al* (2001), Yanovsky and Kay (2003) and Valverde *et al* (2004)
PHYTOCHROME A (PHYA)	Red/far-red light photoreceptor involved in the post-transcriptional regulation of CO	Yanovsky and Kay (2002), Johnson *et al* (1994) and Valverde *et al* (2004)
PHYTOCHROME B (PHYB)	Red light photoreceptor regulating the degradation of CO protein at dawn	Guo *et al* (1998), Yanovsky and Kay (2002), Cerdan and Chory (2003) and Valverde *et al* (2004)
FLAVIN-BINDING, KELCH REPEAT, F-BOX (FKF1)	Photoreceptor required to increase *CO* transcription at dusk	Imaizumi *et al* (2003)

earlier under short days than wild-type plants (Somers *et al*, 1998; Mizoguchi *et al*, 2002). LHY and CCA1 were proposed to act along with TOC1 in a transcriptional feedback loop in which TOC1, which is expressed only in the evening, promotes the expression of *LHY/CCA1* at dawn, and in turn LHY/CCA1 repress the expression of *TOC1* (Alabadi *et al*, 2001). In contrast, *ELF3* does not appear to encode a central component of the circadian clock but modulates light signalling to the oscillator, so that exposure of *elf3* mutants to continuous light or long photoperiods stops circadian rhythms (McWatters *et al*, 2000; Hicks *et al*, 2001; Liu *et al*, 2001b).

Mutations that reduce day-length responses by delaying flowering under long days define a set of circadian-clock-regulated genes. These include the *CONSTANS* (*CO*), *GIGANTEA* (*GI*) and *FLOWERING LOCUS T* (*FT*) genes, which were initially placed in the same genetic pathway based on their mutant phenotypes and the genetic interactions between the mutations (Koornneef *et al*, 1991). All of these genes have now been cloned (Table I), and are circadian clock regulated. Transgenic overexpression of each of the genes in this group causes early flowering (Kardailsky *et al*, 1999; Kobayashi *et al*, 1999; Borner *et al*, 2000; Lee *et al*, 2000; Onouchi *et al*, 2000; Samach *et al*, 2000).

Analysis of the effects of mutant alleles or transgenes on the expression of genes within the pathway allowed their order of action to be determined (Figure 1). Mutations in *LHY/CCA1* and *TOC1* affect the temporal pattern of expression of later-acting genes such as *GI*, *CO* and *FT* (Suarez-Lopez *et al*, 2001; Blazquez *et al*, 2002; Mizoguchi *et al*, 2002; Yanovsky and Kay, 2002). The expression of *GI* is regulated by *LHY/CCA1*, so that in the *lhy cca1* double mutant the timing of expression of *GI* occurs 4 h earlier under long-day conditions (Mizoguchi *et al*, 2002). The major effect of *gi* mutations on flowering appears to be through the regulation of *CO* mRNA levels, because in *gi* mutants these are reduced and

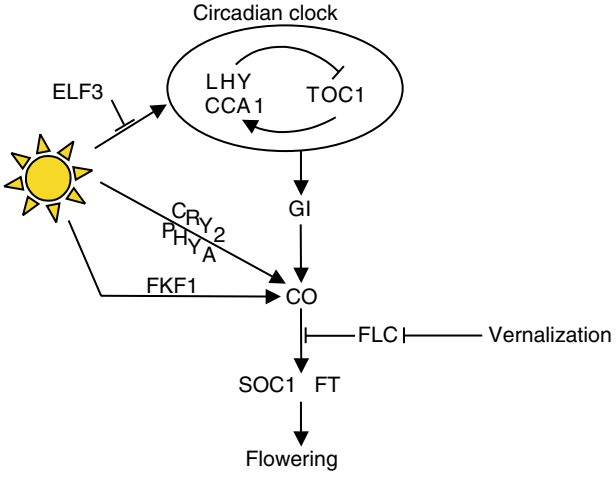

Figure 1 Molecular hierarchy that controls flowering of *Arabidopsis* in response to photoperiod. Arrows between genes represent promotive effects, whereas perpendicular lines represent repressive effects.

the overexpression of *CO* in a *gi* mutant overcomes the late-flowering phenotype (Suarez-Lopez *et al*, 2001). However, *gi* mutations cause additional defects inducing circadian rhythms to cycle faster under constant conditions, and impairing red-light signalling from phytochrome B (Park *et al*, 1999; Huq *et al*, 2000), and the relationship of these effects to the flowering phenotype is unclear. CO activates the expression of the downstream genes *FT* and *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CO 1*) (Kardailsky *et al*, 1999; Kobayashi *et al*, 1999; Borner *et al*, 2000; Lee *et al*, 2000; Onouchi *et al*, 2000; Samach *et al*, 2000). The hierarchy of gene action in the pathway suggested that the early-flowering phenotypes caused by loss-of-function mutations of *elf3*, *lhy*, *cca1* and *toc1* may be largely due to alterations in the timing

of expression of these circadian-clock-regulated genes that control flowering time, particularly *CO* (discussed later) (Strayer *et al*, 2000; Suarez-Lopez *et al*, 2001; Yanovsky and Kay, 2002).

FT and *SOC1* are among the most potent activators of flowering, so that they cause extreme early flowering when overexpressed (Kardailsky *et al*, 1999; Kobayashi *et al*, 1999; Borner *et al*, 2000; Lee *et al*, 2000; Samach *et al*, 2000). Furthermore, in addition to the response to photoperiod, these genes are also regulated by other environmental conditions that influence flowering time, such as exposure of plants to low temperatures for extended durations that mimic winter conditions (vernalization). Therefore, *FT* and *SOC1* are at the point of convergence of several flowering-time pathways and are often therefore described as floral integrators (Mouradov *et al*, 2002; Simpson and Dean, 2002).

A coincidence model for CONSTANS activation by photoreceptors

CO plays a central role in the photoperiod response pathway by mediating between the circadian clock and the floral regulators FT and SOC1 (Suarez-Lopez *et al*, 2001). Furthermore, *CO* mRNA shows a striking temporal pattern of expression that was proposed to provide a basis for the regulation of the pathway by day length. Under long days the mRNA peaks in the evening and stays high until the following dawn, whereas under short days the mRNA peaks during the night (Figure 2) (Suarez-Lopez *et al*, 2001). This suggested that post-transcriptional regulation of *CO* by light specifically under long days might be responsible for the activation of CO, and thereby the response to long days. That the exact timing of CO expression is important in distinguishing between long and short days was also suggested by experiments in which the temporal pattern of *CO* expression was altered using mutants or by altering the length of the daily cycle from 24 h (Roden *et al*, 2002; Yanovsky and Kay, 2002). The *toc1-1* mutant causes circadian rhythms to cycle faster under constant light, and under short days causes *CO* mRNA abundance to peak earlier. This earlier peak in *CO* mRNA under short days occurs during the photoperiod rather than during the night. Surprisingly, this effect of *toc1-1* appears to be specific to *CO* expression, and does not affect expression of the upstream genes *GI* and *LHY* (Yanovsky and Kay, 2002). Nevertheless, expression of *CO* mRNA during the photoperiod correlates with increased *FT* expression and early flowering under short days, and these effects require *CO* function since they are largely abolished in a *co* mutant.

A second approach to altering the phase of *CO* expression involved changing the duration of the 24 h daily cycle. The timing of expression of *CO* relative to the light–dark transitions could be altered by maintaining the ratio of light to dark within the daily cycle, but extending or shortening the cycle from 24 h to 21 or 30 h (Roden *et al*, 2002; Yanovsky and Kay, 2002). This demonstrated a strong correlation between the expression of CO in the light, increased expression of the downstream gene *FT* and early flowering. Although the *toc1-1* mutation and the alteration in cycle duration are likely to affect the timing of expression of many clock-controlled genes, the striking correlation between *CO* expression during the photoperiod, upregulation of *FT* and early flowering strongly suggested that post-transcriptional regulation of *CO*

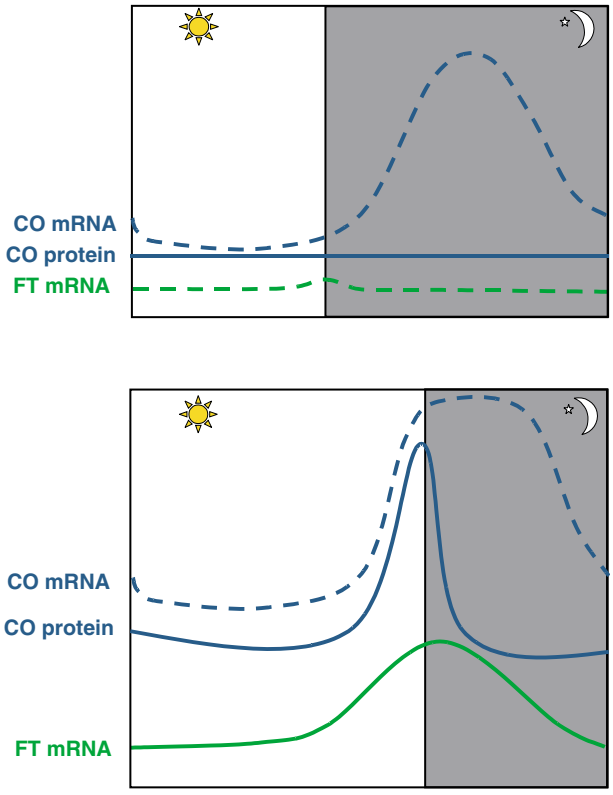

Figure 2 Expression patterns of the mRNAs of circadian-clock-controlled genes *CO* and *FT* under long and short days. Under short days (8 h light:16 h dark), *CO* mRNA expression peaks during the night (upper panel), CO protein does not accumulate and the downstream gene *FT* is not expressed. Under long-day conditions (16 h light:8 h dark), the peak of *CO* mRNA expression partly coincides with light (lower panel), the protein accumulates in the nucleus and the expression of *FT* mRNA is activated. FT promotes early flowering.

by exposure to light is at least one mechanism by which flowering of *Arabidopsis* is activated in response to long days.

Two molecular mechanisms underlying this activation of CO by light were recently described. The stability of the CO protein was shown to be regulated by light, so that in plants exposed to blue or far-red light the protein accumulates in the nucleus, but in darkness or red light the protein is absent (Valverde *et al*, 2004). This correlates with blue and far-red light being the most effective in promoting flowering. Also genetic experiments demonstrate that the blue light photoreceptors cryptochrome 1 and cryptochrome 2 as well as the far-red photoreceptor phytochrome A both promote flowering and stabilize the CO protein, whereas phytochrome B, which is activated by red light, delays flowering and promotes the degradation of CO protein (Johnson *et al*, 1994; Guo *et al*, 1998; El-Assal *et al*, 2001; Yanovsky and Kay, 2002; Cerdan and Chory, 2003; Valverde *et al*, 2004). This post-transcriptional regulation of CO stability by light provides a basis for the original proposal that the coincidence between *CO* mRNA and exposure to light is required to promote flowering.

An independent mechanism based on transcriptional regulation was also recently shown to regulate CO in response to light (Imaizumi *et al*, 2003). Under long days, the broad peak in CO mRNA is biphasic with one peak occurring in the light prior to dusk and a second during the night. This first peak in

mRNA abundance, which facilitates the coincidence between *CO* expression and light, requires exposure to light. The photoreceptor FKF1 (Table I) is required for the expression of this peak, and mutations in FKF1 both delay flowering and reduce *CO* expression at dusk (Imaizumi *et al*, 2003).

The responsiveness of CO activity to day length therefore depends on regulation at several levels. Circadian clock control of *CO* transcription underlies the system and restricts *CO* expression to the later part of the day/night cycle. The presence of light during the evening both enhances *CO* transcription and stabilizes the protein in the nucleus ensuring activation of the floral regulator FT. This requirement for light ensures that CO activation and flowering only occur under long days.

Interactions between the vernalization and photoperiod responses

Natural accessions of *Arabidopsis* differ in their responses to seasonal cues of day length and temperature. Summer annual accessions germinate in spring or early summer and rapidly flower in response to the long-day photoperiod. In contrast, winter annuals typically germinate in summer, grow vegetatively through the winter until the following spring and then flower in response to exposure to long photoperiods the following summer. Thus winter annuals do not respond to inductive photoperiods in the first summer, but require exposure to cold winter temperatures before they can respond to long days the following summer. Winter and summer annuals typically differ at one of two loci, *FLOWERING LOCUS C* (*FLC*) and *FRIGIDA* (*FRI*), and dominant alleles at these loci in the winter annual are required to confer a vernalization requirement (Simpson and Dean, 2002).

FLC encodes a MADS box transcription factor that is expressed at high levels in winter annuals before vernalization, and at lower levels when plants are exposed to cold temperatures for several weeks (Michaels and Amasino, 1999; Sheldon *et al*, 1999). In addition, overexpression of *FLC* in summer annual varieties causes a dramatic late-flowering phenotype. Therefore, *FLC* encodes a repressor of flowering, and high FLC levels correlate with the vernalization requirement of winter annual varieties (Michaels and Amasino, 1999; Sheldon *et al*, 1999). *FRI* encodes a protein with unknown biochemical function (Johanson *et al*, 2000) and is required to increase *FLC* mRNA abundance (Michaels and Amasino, 1999; Sheldon *et al*, 1999). This effect is dependent on functional *FLC* alleles as loss-of-function *flc* mutations suppress the effect of *FRI* on flowering time.

The photoperiod and vernalization pathways respond to different environmental signals, but these pathways converge to regulate the expression of the same downstream genes, *FT* and *SOC1* (Borner *et al*, 2000; Lee *et al*, 2000; Samach *et al*, 2000; Michaels and Amasino, 2001). Transcription of *FT* and *SOC1* is activated by CO and repressed by FLC, which represses *SOC1* transcription by directly binding to its promoter (Hepworth *et al*, 2002). Therefore, in winter annual accessions, response to long days is prevented during the first summer, at least in part because high FLC levels block the capacity of CO to activate downstream genes.

Many other plant species show similar genetic variation between summer and winter annual forms (Laurie, 1997), and must also block the day-length response until they have been exposed to winter conditions. *CO* function is conserved (Yano *et al*, 2000; Griffiths *et al*, 2003) in distantly related species, raising the possibility that antagonism between *CO* and *FLC* orthologues may be the general basis of the winter annual form. No *FLC* orthologues, however, have been identified outside the Cruciferae, suggesting that the role of *FLC* in the vernalization response may not be widely conserved. *VRN1* is required in winter wheat varieties to confer a vernalization response. A candidate for the *VRN1* gene was recently cloned and encodes a MADS box protein most similar to APETALA1 (AP1) from *Arabidopsis* (Yan *et al*, 2003). *vrn1* mutants contain a deletion in the promoter region, suggesting that a negative regulator can no longer repress *VRN1* expression prior to vernalization and this lack of repression promotes flowering. This is consistent with the observation of low *VRN1* mRNA levels before vernalization in winter wheat varieties and increases in its mRNA levels after vernalization (Yan *et al*, 2003). Therefore, no repressor analogous to FLC has been described in monocotyledonous plants, and how the photoperiod response is prevented prior to vernalization remains unclear.

Diversity in photoperiodic responses

Control of flowering by photoperiod is widespread in the plant kingdom, but the type of response can vary widely between species (Thomas and Vince-Prue, 1997). For example, short-day plants flower early under short days and late under long days, and therefore show the reverse response to *Arabidopsis*. The distinction between long- and short-day response types has evolved independently in different families of flowering plants. The grasses include the long-day response plants wheat and barley as well as the short-day response plants maize and rice, while in *Nicotiana*, a single genus of dicotyledonous plants, long- and short-day response types occur. Therefore, whether the molecular pathway described in *Arabidopsis* is conserved in species showing responses to short days is of importance, since this would enable analysis of how the pathway is modified to generate a short-day response and whether these modifications are the same in different branches of the Angiosperm phylogeny.

Genetic analysis of photoperiod response in rice has provided evidence that even in short-day plants distantly related to *Arabidopsis*, the same components regulate photoperiod response. By identifying natural allelic variation affecting photoperiodic control of flowering, the rice orthologues of *CO* (*Hd1* in rice) and *FT* (*Hd3a* in rice) were shown to be required for flowering in response to short days (Yano *et al*, 2000; Kojima *et al*, 2002). *Hd3a* is expressed at higher levels under short days, which induce flowering, and therefore is similar to the transcriptional upregulation of *FT* detected under long days in *Arabidopsis*. Thus, transcriptional upregulation of *FT*/*Hd3a* specifically under day lengths that induce flowering is conserved in both species (Izawa *et al*, 2002; Hayama *et al*, 2003). Furthermore, *Hd1* and the rice orthologue of *GI* (*OsGI*) regulate the expression of *Hd3a* mRNA in rice. However, the relationship between *Hd1* and *Hd3a* appears to be reversed, with elevated *Hd1* causing a reduction in *Hd3a* expression. This relationship between *Hd1* and *Hd3a* suggests that the role of *Hd1* is to repress *Hd3a* under long days and that this repression is relieved in short

days leading to an upregulation of *Hd3a* and early flowering (Hayama *et al*, 2003). Therefore, although both *Arabidopsis* and rice *CO*/*Hd1* regulate *FT*/*Hd3a* expression, their relationships are reversed with CO activating *FT* whereas Hd1 represses *Hd3a*.

Whether a similar mechanism operates in other short-day plants remains to be tested, but at least the components have been shown to occur in other species. *CO*-like genes have been cloned from the short-day plant *Pharbitis nil* (Liu *et al*, 2001a; Kim *et al*, 2003), which is closely related to the *Nicotiana* species, and show a similar pattern of diurnal regulation to *CO* from *Arabidopsis*. Also, Maryland Mammoth tobacco, which shows an absolute requirement for exposure to short days to flower, will flower under long days if it carries a transgene driving constitutive expression of the *Sinapis alba* orthologue of *SOC1*. This suggests that related MADS box proteins act downstream of the photoperiod response in *Arabidopsis* and short-day tobacco varieties (Borner *et al*, 2000).

In addition to flowering, other developmental transitions including tuberization in potato and the onset of dormancy in the buds of perennial plants such as deciduous trees are controlled by day length (Thomas and Vince-Prue, 1997). Some of the proteins identified as regulating photoperiodic control of flowering in *Arabidopsis* may also regulate these other responses. Tuberization of potato is induced by short days, and transgenic plants overexpressing the *Arabidopsis CO* gene showed delayed tuberization, suggesting that potato orthologues of CO may negatively regulate tuberization (Martinez-Garcia *et al*, 2002).

At present our understanding of these processes is largely at the genetic level, there is a need to extend this to include the biochemical basis of the response in *Arabidopsis*, and how the biochemical function of the constituent proteins is altered to generate other photoperiodic response types. This will provide an understanding of how the activity of this pathway has been modified during evolution and provide insights into how the flowering time of crop or horticultural plants could be modified to generate novel flowering phenotypes.

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References

Alabadi D, Oyama T, Yanovsky MJ, Harmon FG, Mas P, Kay SA (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the *Arabidopsis* circadian clock. *Science* **293:** 880–883

Alabadi D, Yanovsky MJ, Mas P, Harmer SL, Kay SA (2002) Critical role for CCA1 and LHY in maintaining circadian rhythmicity in *Arabidopsis*. *Curr Biol* **12:** 757–761

Blazquez MA, Trenor M, Weigel D (2002) Independent control of gibberellin biosynthesis and flowering time by the circadian clock in *Arabidopsis*. *Plant Physiol* **130:** 1770–1775

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

Bünning E (1936) Die endogene Tagesrhythmik als Grundlage der photoperiodischen Reaktion. *Ber Dtsch Bot Ges* **54:** 590–607

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

Dowson-Day MJ, Millar AJ (1999) Circadian dysfunction causes aberrant hypocotyl elongation patterns in *Arabidopsis*. *Plant J* **17:** 63–71

El-Assal SED, Alonso-Blanco C, Peeters AJM, Raz V, Koornneef M (2001) A QTL for flowering time in *Arabidopsis* reveals a novel allele of *CRY2*. *Nat Genet* **29:** 435–440

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

Garner WW, Allard HA (1920) Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. *J Agric Res* **18:** 553–606

Griffiths S, Dunford RP, Coupland G, Laurie DA (2003) The evolution of CONSTANS-like gene families in barley, rice, and *Arabidopsis*. *Plant Physiol* **131:** 1855–1877

Guo HW, Yang WY, Mockler TC, Lin CT (1998) Regulations of flowering time by *Arabidopsis* photoreceptors. *Science* **279:** 1360–1363

Harmer SL, Hogenesch LB, Straume M, Chang HS, Han B, Zhu T, Wang X, Kreps JA, Kay SA (2000) Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. *Science* **290:** 2110–2113

Hayama R, Coupland G (2003) Shedding light on the circadian clock and the photoperiodic control of flowering. *Curr Opin Plant Biol* **6:** 13–19

Hayama R, Yokoi S, Tamaki S, Yano M, Shimamoto K (2003) Adaptation of photoperiodic control pathways produces short-day flowering in rice. *Nature* **422:** 719–722

Hepworth SR, Valverde F, Ravenscroft D, Mouradov A, Coupland G (2002) Antagonistic regulation of flowering-time gene SOC1 by CONSTANS and FLC via separate promoter motifs. *EMBO J* **21:** 4327–4337

Hicks KA, Albertson TM, Wagner DR (2001) EARLY FLOWERING3 encodes a novel protein that regulates circadian clock function and flowering in *Arabidopsis*. *Plant Cell* **13:** 1281–1292

Huq E, Tepperman JM, Quail PH (2000) GIGANTEA is a nuclear protein involved in phytochrome signaling in *Arabidopsis*. *Proc Natl Acad Sci USA* **97:** 9789–9794

Imaizumi T, Tran HG, Swartz TE, Briggs WR, Kay SA (2003) FKF1 is essential for photoperiodic-specific light signalling in *Arabidopsis*. *Nature* **426:** 302–306

Izawa T, Oikawa T, Sugiyama N, Tanisaka T, Yano M, Shimamoto K (2002) Phytochrome mediates the external light signal to repress FT orthologs in photoperiodic flowering of rice. *Genes Dev* **16:** 2006–2020

Johanson U, West J, Lister C, Michaels S, Amasino R, Dean C (2000) Molecular analysis of FRIGIDA, a major determinant of natural variation in *Arabidopsis* flowering time. *Science* **290:** 344–347

Johnson E, Bradley M, Harberd NP, Whitelam GC (1994) Photoresponses of light-grown phyA mutants of *Arabidopsis*: phytochrome A is required for the perception of daylength extensions. *Plant Physiol* **105:** 141–149

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

Kim SJ, Moon J, Lee I, Maeng J, Kim SR (2003) Molecular cloning and expression analysis of a CONSTANS homologue, PnCOL1, from *Pharbitis nil*. *J Exp Bot* **54:** 1879–1887

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

Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M (2002) Hd3a, a rice ortholog of the *Arabidopsis* FT gene, promotes transition to flowering downstream of Hd1 under short-day conditions. *Plant Cell Physiol* **43:** 1096–1105

Koornneef M, Hanhart CJ, Van Der Veen JH (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol Gen Genet* **229:** 57–66

Laurie DA (1997) Comparative genetics of flowering time. *Plant Mol Biol* **35:** 167–177

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

Liu JY, Yu JP, McIntosh L, Kende H, Zeevaart JAD (2001a) Isolation of a CONSTANS ortholog from *Pharbitis nil* and its role in flowering. *Plant Physiol* **125:** 1821–1830

Liu XL, Covington MF, Fankhauser C, Chory J, Wanger DR (2001b) ELF3 encodes a circadian clock-regulated nuclear protein that functions in an *Arabidopsis* PHYB signal transduction pathway. *Plant Cell* **13:** 1293–1304

Martinez-Garcia JF, Virgos-Soler A, Prat S (2002) Control of photoperiod-regulated tuberization in potato by the *Arabidopsis* flowering-time gene CONSTANS. *Proc Natl Acad Sci USA* **99:** 15211–15216

McWatters HG, Bastow RM, Hall A, Millar AJ (2000) The ELF3 zeitnehmer regulates light signalling to the circadian clock. *Nature* **408:** 716–720

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

Michaels SD, Amasino RM (2001) Loss of FLOWERING LOCUS C activity eliminates the late-flowering phenotype of FRIGIDA and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell* **13:** 935–941

Mizoguchi T, Wheatley K, Hanzawa Y, Wright L, Mizoguchi M, Song HR, Carre IA, Coupland G (2002) LHY and CCA1 are partially redundant genes required to maintain circadian rhythms in *Arabidopsis*. *Dev Cell* **2:** 629–641

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

Onouchi H, Igeno MI, Perilleux C, Graves K, Coupland G (2000) Mutagenesis of plants overexpressing CONSTANS demonstrates novel interactions among *Arabidopsis* flowering-time genes. *Plant Cell* **12:** 885–900

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

Pittendrigh CS, Minis DH (1964) The entrainment of circadian oscillations by light and their role as photoperiodic clocks. *Am Nat* **98:** 261–322

Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The CONSTANS gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* **80:** 847–857

Redei GP (1962) Supervital mutants of *Arabidopsis*. *Genetics* **47:** 443–460

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

Roden LC, Song HR, Jackson S, Morris K, Carre IA (2002) Floral responses to photoperiod are correlated with the timing of rhythmic expression relative to dawn and dusk in *Arabidopsis*. *Proc Natl Acad Sci USA* **99:** 13313–13318

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

Schaffer R, Landgraf J, Accerbi M, Simon V, Larson M, Wisman E (2001) Microarray analysis of diurnal and circadian-regulated genes in *Arabidopsis*. *Plant Cell* **13:** 113–123

Schaffer R, Ramsay N, Samach A, Corden S, Putterill J, Carre IA, Coupland G (1998) The late elongated hypocotyl mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering. *Cell* **93:** 1219–1229

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

Simpson GG, Dean C (2002) *Arabidopsis*, the Rosetta stone of flowering time? *Science* **296:** 285–289

Somers DE, Webb AAR, Pearson M, Kay SA (1998) The short-period mutant, toc1-1, alters circadian clock regulation of multiple outputs throughout development in *Arabidopsis thaliana*. *Development* **125:** 485–494

Strayer C, Oyama T, Schultz TF, Raman R, Somers DE, Mas P, Panda S, Kreps JA, Kay SA (2000) Cloning of the *Arabidopsis* clock gene TOC1, an autoregulatory response regulator homolog. *Science* **289:** 768–771

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

Thomas B, Vince-Prue B (1997) *Photoperiodism in Plants*, 2nd edn. San Diego, CA: Academic Press

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

Wang Z-Y, Tobin EM (1998) Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. *Cell* **93:** 1207–1217

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of wheat vernalization gene VRN1. *Proc Natl Acad Sci USA* **100:** 6263–6268

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene CONSTANS. *Plant Cell* **12:** 2473–2483

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

Yanovsky MJ, Kay SA (2003) Living by the calendar: how plants know when to flower. *Nat Rev Mol Cell Biol* **4:** 265–275

