Flowering in time: genes controlling photoperiodic flowering in *Arabidopsis*

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Successful sexual reproduction in plants relies upon the strict coordination of flowering time with favourable seasons of the year. One of the most important seasonal cues for the model plant *Arabidopsis thaliana* (*Arabidopsis*) is day length. Genes influencing flowering time in *Arabidopsis* have been isolated, some of which are involved in the perception and signalling of day length. This review discusses recent progress that has been made in understanding how *Arabidopsis* integrates environmental and internal signals to ensure a sharp transition to flowering and new insights on the role of the circadian clock in controlling the expression of genes that promote flowering in response to day length.

Keywords: flowering time genes; circadian clock; *Arabidopsis*; day length; photoperiodic

1. INTRODUCTION

The growth, development and reproduction of many organisms are synchronized to favourable seasons of the year. In plants, one of the most important of these seasonal processes is flowering. Day length (photoperiod) and temperature are commonly used as seasonal signals. The long days (LDs) of spring and summer are sufficient to accelerate flowering in some varieties of the model plant *Arabidopsis*. In other *Arabidopsis* varieties, rapid flowering also requires long periods of low temperature (vernalization) during the winter ensuring that they will not flower until spring.

Over 80 genes affecting *Arabidopsis* flowering time (flowering time genes) have been identified by mutation and these have been placed into different floral promotive pathways by genetic and physiological analyses (figure 1). Genes involved in day length perception and transduction, including genes that regulate circadian clock function, are in the LD pathway. Those controlling the perception and response to cold temperatures have been placed in the vernalization pathway. Two other pathways contain genes that promote flowering independently of environmental signals. The autonomous pathway synthesizes or responds to internal factors produced as the plant ages to promote flowering. The gibberellic acid (GA) pathway produces and transduces a GA signal required for flowering in non-inductive conditions such as short days (SDs). Finally, genes involved in light quality perception and transduction influence both the LD and autonomous flowering-time pathways. The pathways all act ultimately by upregulating the floral meristem identity genes needed for floral development (see recent comprehensive reviews by Koornneef *et al.* 1998; Simpson *et al.* 1999).

The focus of this review is first on recent work in *Arabidopsis* that begins to show how light signals are perceived and transduced *via* circadian regulated genes

into responses such as flowering. Second, recent discoveries of points of convergence of different flowering time pathways in *Arabidopsis* are discussed briefly.

2. PERCEPTION OF DAY LENGTH

Several models have been proposed to explain how the perception of day length leads to control of developmental responses such as flowering. The most prominent is the external coincidence model, which proposes the coincidence of an external signal, light, with an internally regulated photo-inducible phase, maintained at the same time each day by the circadian clock (for a review, see Thomas & Vince-Prue 1997). This coincidence with light delays flowering in SD plants or promotes flowering in LD plants. A second model, the internal coincidence model, proposes the coincidence of two circadian rhythms in inductive conditions that promote flowering.

Evidence for the role of the circadian clock in day length perception comes from experiments where extended dark periods are broken with light applications (light breaks). Plants show a rhythmic pattern in their flowering response in sensitivity to these light pulses, where applications of light at particular times influence flowering but at other times have no effect, thus implying that the underlying oscillator is driving the sensitivity cycle (for a review, see Thomas & Vince-Prue 1997).

Studies of LD plants suggest that as well as timing, the duration, intensity and wavelength of light are all important for LD promotion of flowering. This led Thomas & Vince-Prue (1997) to propose a modified external coincidence model for LD plants. In this model, there is an internally regulated rhythm in sensitivity to far red light. Extended red light breaks (or LD) either directly interact with the rhythm and/or rephase it, so that the peak in far red sensitivity coincides with the light period and flowering is promoted.

Figure 1. Outline of the four main flowering-time pathways in *Arabidopsis* and the genes at which they converge. Lines with arrowheads indicate interactions between genes or promotive effects on gene expression and flowering. Lines with bars indicate repressive effects on gene expression or flowering. The genes indicated are described in the text. Briefly, the *CCA1* and *LHY* genes are closely involved with circadian clock function. Expression of the *GI* and *CO* genes is under circadian clock control and, along with *FT*, these genes promote flowering predominantly in long day conditions (long day pathway). *FCA* promotes flowering independently of the environment (autonomous pathway). *FCA* appears to exert its effect mainly by regulating the levels of the floral repressor *FLC*, which is also under the control of the vernalization pathway. *SOC1* has been recently identified by mutation, promotes flowering independently of day length and, along with *FT*, is a target of both the long day and autonomous pathways.

3. LIGHT AND PHOTOPERIODIC FLOWERING

In *Arabidopsis*, combinations of the red and far red absorbing photoreceptors phytochromes (PHYs) A to E and the blue light receptors cryptochromes (CRYs) 1 and 2, are involved in the perception of colour, intensity, duration and periodicity of light. Analysis of light signalling in plants suggests that a web of synergistic and antagonistic interactions between photoreceptors, light signalling pathways and the circadian clock regulate plant germination, growth and development (for reviews, see Deng & Quail 1999; Neff *et al.* 2000).

Standard LD conditions (16 h white light, 8 h dark) reveal a role for CRY2 in floral promotion in LD and for PHYB in repressing flowering (for a review, see Lin 2000). Experiments with different wavelengths of constant light show that far red and blue light promote *Arabidopsis* flowering, while constant red light is inhibitory. Similar experiments with mutants suggest that CRY2 may in part promote flowering by antagonizing the inhibitory effect of PHYB in red light on flowering (for a review, see Lin 2000). Promotion of flowering in constant blue light in some ecotypes requires CRY-1 activity, while the promotive effect of constant far red on flowering seems to depend on PHYB and other phytochromes (for a review, see Lin 2000).

The circadian clock is an internal pacemaker that controls many rhythmic processes that occur with a period of ca. 24 h (circadian rhythms). The identification of the circadian clock mechanism is most advanced in *Drosophila* and *Neurospora* where rhythms are generated by proteins such as PERIOD, TIMELESS and FREQUENCY whose amounts rise and fall in a daily cycle. These proteins participate in a negative feedback loop, cyclically regulating their own transcription (Dunlap 1999). The clock is synchronized to the external environment by light and the phase (timing) of circadian rhythms, either advance or delay, depending on the time at which light is given. In *Drosophila*, these light pulses change the levels of light-labile TIMELESS protein, while in *Neurospora* the clock is reset by induction of *FREQUENCY* transcription by light (for a review, see Dunlap 1999).

In *Arabidopsis*, the molecular bases of the clock and of phase shifting by light are not well understood. However, the photoreceptors responsible for sending light signals that regulate the period of circadian rhythms were analysed using photoreceptor mutants. The work suggests that combinations of *Arabidopsis* photoreceptors send light signals to the clock. PHYB is most important in high intensity red light, PHYA in low intensity red or blue light and CRY-1 in high or low intensity blue light (Millar *et al.* 1995b; Somers *et al.* 1998a).

In *Arabidopsis*, flowering is promoted by exposing plants to night interruptions (light breaks). PHYA was partly responsible for detection of white light breaks in *Arabidopsis* (Reed *et al.* 1994). Light breaks of all wavelengths tested promoted flowering in wild-type, with far red being most effective (Goto *et al.* 1991). *Arabidopsis* responds best to far red given in the middle of the night, suggesting changing sensitivity to far red through the daily cycle, as in many other LD plants (Goto *et al.* 1991). Sensitivity to far red appears to be under circadian clock control in *Arabidopsis* as there was a rhythmic flowering response to far red given at different times during constant white light (Deitzer 1984). PHYA is also implicated in photoperiodic flowering in day length extension experiments. *Arabidopsis* was grown in short days (8 h white light), followed by 8 h low fluence rate incandescent light, which is high in far red but photosynthetically inactive. Light enriched with far red typifies natural twilight conditions (ratio red:far red = 0.65:1.15) (Neff *et al.* 2000). Sensing of the day extensions is mediated in part by PHYA, because *phya* mutants flower considerably later than wild-type plants in these conditions (Johnson *et al.* 1994).

PHYA may exert its effect on photoperiodic flowering by having a direct effect on the expression or activity of flowering-time genes (Samach & Coupland 2000), but this needs to be tested. Alternatively, PHYA may also influence photoperiodic flowering by resetting the circadian clock, because high intensity far red given at the end of the night period rephased the rhythm of leaf movement in wild-type *Arabidopsis* but not in *phya* mutants (Yanovsky *et al.* 2000). Thus it will be interesting to determine whether the light breaks given in the middle of the night that promote flowering in *Arabidopsis* also rephase circadian rhythms.

Recent advances in the understanding of the early steps in light signalling suggest that phytochromes can directly

regulate gene expression by binding to transcription factors in the nucleus. PHYA is discussed here given its role in day length perception in *Arabidopsis*. Far red light causes a functional PHYA:green fluorescent protein fusion protein to congregate in the cytoplasm in spots, or to move to the nucleus in transgenic *Arabidopsis* plants (Kim *et al.* 2000). Furthermore, in pea, immunolocalization experiments with PHYA antibodies indicate that the P_r form of PHYA, which forms after exposure to far red light, is reasonably stable in the nucleus (Hisada *et al.* 2000). Nuclear and cytoplasmic components of the PHYA signalling pathway have also been identified. These include proteins that interact with PHYA in yeast two hybrid screens (e.g. the bHLH transcription factor PIF3), targets for phosphorylation by PHYA (e.g. the cytoplasmic protein PKS1), and genes identified in genetic screens as either positive or negative regulators of PHYA signalling (e.g. *FHY1* and *SPA1*) (reviewed by Deng & Quail 1999; Neff *et al.* 2000).

Furthermore, the PHYA and PHYB signalling pathways overlap and PHYB, in its active P_{fr} form, has been shown to bind PIF3 (Ni *et al.* 1999). In gel retardation assays, PIF3 binds the promoter G box present in some light-regulated genes including *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), and PHYB interacts with the PIF3/DNA complex in a red light-dependent fashion (Martinez-Garcia *et al.* 2000). The *CCA1* gene was first identified as a transcription factor needed for phytochrome-mediated upregulation of the light-regulated photosynthetic gene *CAB2* (*CHLOROPHYLL A/B BINDING PROTEIN 2*, also known as *LHCBI*1*) (*CAB2*) (Wang *et al.* 1997). Subsequent work showed that *CCA1* also functions to regulate circadian rhythms in *Arabidopsis* (see § 4).

These results suggest how light-activated phytochromes might directly activate genes such as *CCA1* to regulate downstream light-regulated genes (e.g. *CAB*) and simultaneously influence circadian rhythms. Transduction of the light signals that promote flowering, *via* circadian regulated flowering-time genes, is discussed in § 4.

4. THE CIRCADIAN CLOCK AND PHOTOPERIODIC FLOWERING

Good evidence for the role of the plant circadian clock in flowering has come with the isolation of *Arabidopsis* mutants with both altered circadian rhythms and flowering time and with the identification of circadian-regulated flowering time genes (for reviews, see Millar 1999; Somers 1999; Samach & Coupland 2000; McClung 2000).

Screens for *Arabidopsis* mutants with longer or shorter circadian periods were carried out using a bioluminescence assay with the *CAB2::luciferase* reporter gene, which cycles with a peak in the morning (Millar *et al.* 1995a). For example, the screen identified a short period mutant *toc1*, which confers photoperiod insensitive flowering in some *Arabidopsis* varieties (Somers *et al.* 1998b; Somers, 2001). Photoperiod sensitivity can be restored by changing the timing of the LD cycles to conform to the period of the internal clock in *toc1*, indicating that the period length lesion is responsible for the altered flowering time (Strayer *et al.* 2000). The screen also identified *ZEITLUPE* (*ZTL*) which appears to be involved in light

input to the clock (Somers *et al.* 2000; Somers, 2001). A second *ZTL*-related gene *FKF* also influences flowering time and circadian rhythms (Nelson *et al.* 2000).

Several *Arabidopsis* mutants are arrhythmic and have altered flowering. These include the *early flowering 3* (*elf3*) mutant identified by its early day length-insensitive flowering and found to be arrhythmic in continuous light conditions. The *ELF3* gene is thought to be involved in gating (restricting) light signalling to the clock (Zagotta *et al.* 1996; Hicks *et al.* 1996; McWatters *et al.* 2001).

CCA1 encodes a transcription factor with a single MYB repeat, and overexpression of the gene causes arrhythmia and late flowering, while a loss of function mutation leads to a short period phenotype (Wang & Tobin 1998; Green & Tobin 1999). A related MYB gene *LATE ELONGATED HYPOCOTYL* (*LHY*) also causes arrhythmia and late flowering when overexpressed in the *lhy* mutant (Schaffer *et al.* 1998). *CCA1* and *LHY* negatively regulate their own expression, suggesting that they may be central clock components or involved in light input to the clock. *CCA1* may also be considered an output component as it binds directly to the *CAB* promoter (Wang *et al.* 1997). *CCA1* and *LHY* appear to have both overlapping and non-redundant functions, the extent of which should be revealed by analysis of a *lhy* loss of function mutant and the double *cca1 lhy* mutant. Recently, a molecular model was proposed for the autoregulatory feedback loop making up the plant circadian clock that has the *TOC1*, *CCA1* and *LHY* genes as key components (Albadini *et al.* 2001).

Mutations in the LD pathway genes *GIGANTEA* (*GI*) and *CONSTANS* (*CO*) delay flowering under LD but have little or no effect under SD. Hence both genes are thought to play an important role in promoting flowering in LD (Koornneef *et al.* 1991, 1998). Strikingly, expression of both *GI* and *CO* is under circadian clock control and has a distinct wave form in different day lengths (Fowler *et al.* 1999; Park *et al.* 1999; Suarez-Lopez *et al.* 2001). *GI* transcript levels peak 8–10 h into light. The peak in LD is later, slightly lower, but of longer duration in the light period than in SD. The peak of *CO* transcript levels coincides with light in LD but not in SD.

High *CO* levels are known to promote flowering (Putterill *et al.* 1995; Onouchi *et al.* 2000). *GI* transcript levels are high at all times in *elf3* mutants (Fowler *et al.* 1999). The late flowering phenotype of *gi elf3* double mutants indicates that *GI* is indeed required for rapid flowering of *elf3* (Chou & Yang 1999). In contrast to the *elf3* mutant, expression of the *GI* gene damps to high levels in the *CCA1* overexpressor, but the plants are late flowering, suggesting that high levels of *GI* are not sufficient to promote flowering in this background (Fowler *et al.* 1999). *GI* levels are very low in the late-flowering *lhy* mutant (Fowler *et al.* 1999; Park *et al.* 1999).

These aspects of gene expression suggest that rapid flowering may occur in LD because: (i) LD rephases the rhythm of *GI/CO* or other flowering-time gene expression, leading to a (ii) high level of transcription or gene activity due to the coincidence with light (external coincidence). In addition, it is possible that coincident expression of flowering time genes with each other, or with other internal factors in LD, may be required to promote

Figure 2. Model of the role of circadian-regulated flowering-time genes in photoperiodic control of flowering in *Arabidopsis*. In LD, light rephases and alters the waveform of circadian-regulated genes. This leads to coincident expression of genes with light (external coincidence) or possibly with each other (internal coincidence) to promote flowering.

flowering (internal coincidence) (Samach & Coupland 2000) (figure 2).

How is the LD signal transduced *via GI* and *CO* to promote flowering? *GI* is a large, novel protein that is nuclear localized in transient expression assays, but little is known of its molecular function (Fowler *et al.* 1999; Park *et al.* 1999; Huq *et al.* 2000). *GI* has been suggested to be upstream of *CO* because a *35S::CO* construct (over-expressing *CO*) restores early flowering to *gi* mutants (for a review, see Samach & Coupland 2000).

While *GI* expression is controlled by the clock, it also appears to be part of a feedback loop that affects amplitude, level and period of circadian rhythms (Fowler *et al.* 1999; Park *et al.* 1999). For example, both the *LHY* and *CCA1* genes showed a much reduced level and amplitude of expression in *gi* mutants. *GI* may regulate the circadian clock *via* the light input pathway because there is a reduced response to light on period length in the *gi-1* mutant (Park *et al.* 1999). *gi* mutants also have elongated hypocotyls in white, red and blue light which may reflect a defect in light signalling or altered circadian function (Araki & Komeda 1993; Huq *et al.* 2000; K. Lee, G. Coupland and J. Putterill, unpublished data; Dowson-Day & Millar 1999). This raises the possibility that *GI* might regulate flowering time through altering the period

of circadian rhythms. However, two different *gi* mutant alleles have different effects on the period of circadian regulated gene expression (either shorter or longer periods than wild-type) but both cause late flowering (Park *et al.* 1999). It seems more likely that *GI* exerts its effect on flowering by controlling the level of expression of circadian-regulated genes such as *CO*.

Since *CO* is circadian regulated, but does not affect circadian function in *Arabidopsis* (D. Somers, personal communication), *CO* may be a key link between the circadian clock and control of flowering. *CO* encodes a putative transcription factor with zinc finger motifs and early target genes of *CO* are discussed in § 5.

5. EARLY TARGET GENES OF CO

Targets of *CO* were previously identified using a steroid-inducible *CO* protein (Simon *et al.* 1996). Plants containing the fusion protein of *CO* with the ligand-binding domain of the rat glucocorticoid receptor (35S::CO:GR), were watered with the steroid dexamethasone (Dex) to allow the *CO* fusion protein to be translocated into the nucleus. The key floral meristem identity gene *LEAFY* (*LFY*) (Weigel *et al.* 1992) was shown to be upregulated within 24 h of *CO* activation (Simon *et al.* 1996). A second gene, *FT*, needed for floral promotion and stem elongation, was shown to be upregulated within 12 h using the same construct (Kobayashi *et al.* 1999).

In the most recent study, the procedure was refined to try and identify direct early targets of *CO* (Samach *et al.* 2000). In this regime, the translational inhibitor cycloheximide was included with the Dex application. *FT* was switched on within 2 h, indicating that it is likely to be a direct target of *CO*. *FT* transcript levels are reduced early in development in *co* mutant plants, consistent with action partly downstream of *CO* (Kardailsky *et al.* 1999; Kobayashi *et al.* 1999). Three new gene targets of *CO* were also identified: (i) the MADS transcription factor *AGL20* (re-named *SOC1* for *SUPPRESSOR OF OVEREXPRESSION OF CO1*, see below); (ii) *ACS10*, encoding a putative enzyme involved in ethylene biosynthesis; and (iii) *AtP5CS2*, encoding an enzyme catalysing proline biosynthesis (Samach *et al.* 2000).

Complementary genetic studies provided elegant confirmation of the molecular work implicating *FT* and *SOC1* as *CO* targets. Mutations in *FT*, *SOC1* or a third flowering-time gene, *FWA*, partly delay the flowering of 35S::CO plants, consistent with the idea that these genes are downstream of *CO* (Onouchi *et al.* 2000). *FT* and *FWA* genes are in the LD pathway (Koornneef *et al.* 1991). However, although *SOC1* is a target of *CO*, the *soc1* mutation alone weakly delays flowering in both LD and SD, placing it in the autonomous pathway (Samach *et al.* 2000).

This molecular and genetic evidence firmly places the *SOC1* transcription factor in the pantheon of classical *Arabidopsis* flowering-time genes that promote flowering. Its role in flowering had been previously suspected, as a homologue *MADSA* is expressed during floral induction in the related mustard *Sinapis alba* (Menzel *et al.* 1996). In *Arabidopsis*, the expression of the gene is also partly controlled by day length. It is not expressed in young SD-grown plants, but is expressed in the shoot apical

meristem and leaf primordia after the shift to continuous light (Samach *et al.* 2000).

The molecular characterization of *FT* was reported recently by two groups (Kardailsky *et al.* 1999; Kobayashi *et al.* 1999). Their work highlights several striking features of *FT*. First, *FT* is related to a membrane-associated mammalian protein named phosphatidylethanolamine binding protein that binds hydrophobic ligands and can be cleaved to form a signalling peptide. The human version is identical to Raf Kinase Inhibitor Protein which regulates activity of the kinase signalling RAF/MEK/ERK pathway. Second, *FT* is over 50% identical to another *Arabidopsis* gene, *TFL*, also upregulated by *CO* but with opposite effects on flowering to *FT*. This raises the possibility that the response to flowering signals is partly controlled by the relative levels of *TFL* and *FT*. Finally, *FT*, along with *FWA*, is intriguing because it does not appear to promote flowering by upregulating the transcriptional activity of *LFY* (Nilsson *et al.* 1998). Instead, molecular and genetic evidence suggests that it functions in a parallel pathway to *LFY*, which either affects the activity of the *LFY* protein, or targets of *LFY*, such as the MADS transcription factor *APETALA1* needed for normal floral development (Ruiz-Garcia *et al.* 1997; Kardailsky *et al.* 1999). Consistent with this idea of parallel LD pathways downstream of *CO*, recent work has shown that the delay in flowering of *35S::CO* is much greater with the double mutants *ft soc1* or with *fwa soc1* than with the single mutants alone (Onouchi *et al.* 2000; Samach *et al.* 2000).

6. FLOWERING PATHWAYS CONVERGE

The multiple *Arabidopsis* flowering pathways must link up, either sooner or later, to switch on flower development. Interactions between the pathways were previously suggested by intensive genetic studies with mutants (for a review, see Koornneef *et al.* 1998). Physiological studies also suggested interactions. For example, factors produced as the plant ages made *Arabidopsis* more responsive to LD (Mozley & Thomas 1995). It was also known that genes from three different floral promotion pathways (autonomous pathway, LD pathway and GA dependent pathway) were somehow involved in transcriptional upregulation of *LFY* (Simon *et al.* 1996; Nilsson *et al.* 1998).

The recent study of Blazquez & Weigel (2000) asked whether this regulation was at the level of the *LFY* promoter or acted on genes upstream of *LFY*. By a process of mutagenesis, they were able to identify an upstream element, a potential MYB protein binding site, in the *LFY* promoter that is critical for activity of *LFY* in response to GA. However, LD activity of the promoter was maintained in the mutants carrying a deletion of this box, indicating that it was not needed for LD activity of *LFY*. Therefore, signals from the LD pathway and from the GA pathway were integrated independently at the *LFY* promoter.

A second study, from Samach *et al.* (2000), followed from the work identifying early target genes, *SOC1* and *FT*, of the LD pathway gene *CO*. Both *SOC1* and *FT* were expressed at a lower levels in the *fca* mutant which affects the autonomous pathway (Samach *et al.* 2000). Thus *SOC1* and *FT* are targets of two *Arabidopsis* flowering time pathways, the autonomous pathway and the LD pathway.

The effect of *fca* on *SOC1* and *FT* expression is likely to be via the *FLOWERING LOCUS C (FLC)* gene. *FLC* is a MADS box transcriptional regulator which encodes a floral repressor. It defines another point of convergence of the flowering-time pathways, being both a target of genes in the autonomous pathway and a critical mediator of the vernalization (cold temperature) response in *Arabidopsis* (for a review, see Sheldon *et al.* 2000). Lee *et al.* (2000) provide additional evidence that *SOC1* is a downstream target of *FLC* repression, by showing that overexpression of *SOC1* (by activation tagging) suppresses the late flowering phenotype of plants carrying *FLC*.

These data indicate that three of the pathways to *Arabidopsis* flowering, the LD, autonomous and vernalization pathways, converge at the *SOC1* and *FT* genes and that their levels may be set by the activities of *CO* and *FLC* (see figure 1) (Samach *et al.* 2000). The GA pathway appears to act somewhat independently of the LD pathway, as there is a separate upstream element in the *LFY* promoter for this pathway. However, the GA pathway has also been proposed to interact with *FLC* since a dominant allele of *FLC* caused a reduced response to GA (for a review, see Sheldon *et al.* 2000). Finally, while *FLC* has been placed at the intersection of the autonomous and vernalization pathways, Quantitative Trait Loci (QTLs) for shortened circadian periods map near *FLC* (Swarup *et al.* 1999), suggesting that *FLC* may feedback on or somehow interact with the LD pathway.

7. PERSPECTIVES

Analysis of *Arabidopsis* flowering-time genes is beginning to yield a coherent molecular picture of how environmental and developmental signals are integrated to regulate flowering time. For example, three flowering pathways, the LD, autonomous and vernalization, converge on downstream targets such as *SOC1* and *FT* to promote flowering.

Genetic analysis has also provided strong evidence for the role of the circadian clock in day length responses. Clock and day-length regulation of the flowering-time genes *GI* and *CO* suggest that the coincidence of high levels of these products with light, or with other internal factors, may be important for floral promotion in LD. This idea is being tested experimentally by phasing the expression of flowering-time genes to different times of the daily cycle and measuring the effect on flowering time (R. Milich & J. Putterill, unpublished data). While little is known about light regulation of flowering-time protein activity, levels of the *Arabidopsis* transcriptional activator *HY5* were found to be more abundant in the light due to light inactivation of a proteasome component (Osterlund *et al.* 2000). It will also be very interesting to test if flowering-time proteins are preferentially stabilized or post-translationally activated as a consequence of coincidence with light. Novel proteins needed for correct responses to seasonal changes in day length should be identified using yeast two hybrid experiments to detect interactors with known flowering-time proteins, and timed, global proteomic studies to detect coincident proteins. Similar global analyses of circadian-regulated transcripts have recently been carried out in *Arabidopsis*

for the first time using DNA microarrays (Harmer 2000; Schaffer 2001).

Finally, how well does the *Arabidopsis* flowering-time model translate to other plants? A rice gene, *Hdl*, controlling flowering time in response to day length, has recently been isolated by map-based cloning and is a homologue of the *Arabidopsis* *CO* gene (Yano *et al.* 2000). This is particularly striking as rice is a SD plant, and it raises the possibility that similar pathways may control day length responses in LD and SD plants.

I am very grateful to Paula Suarez-Lopez, Karen Lee, George Coupland, Masahiro Yano, Robert Schaffer, Dave Somers, Stacey Harmer and Steve Kay for sharing data prior to publication, to Guy Warman and Fran Robson for critical reading of the manuscript and to the editor and an anonymous reviewer for helpful comments.

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