Time measurement and the control of flowering in plants

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Summary
Many plants are adapted to flower at particular times of year, to ensure optimal pollination and seed maturation. In these plants flowering is controlled by environmental signals that reflect the changing seasons, particularly daylength and temperature. The response to daylength varies, so that plants isolated at higher latitudes tend to flower in response to long daylengths of spring and summer, while plants from lower latitudes avoid the extreme heat of summer by responding to short days. Such responses require a mechanism for measuring time, and the circadian clock that regulates daily rhythms in behaviour also acts as the timer in the measurement of daylength. Plants from high latitudes often also show an extreme response to temperature called vernalisation in which flowering is repressed until the plant is exposed to winter temperatures for an extended time. Genetic approaches in Arabidopsis have identified a number of genes that control vernalisation and daylength responses. These genes are described and models presented for how daylength might regulate flowering by controlling their expression by the circadian clock. BioEssays 22:38–47, 2000. © 2000 John Wiley & Sons, Inc.

Introduction
Plants and animals that live at high latitudes often alter their behaviour or development in response to the changing seasons. These alterations include the initiation of reproduction in birds, mammals, and plants, the onset of diapause in many insects, variations in wing colour in butterflies, and migratory restlessness in birds. Environmental signals, often changes in daylength and temperature, trigger these responses. The role of daylength in controlling seasonal responses was originally proposed by Garner and Allard(1) when they recognised that daylength controlled the initiation of flowering in many plant species. They showed that some plants would flower only in daily cycles in which the light period was longer than a particular threshold length, referred to as the critical daylength, while other plants would flower only if daylength was shorter than a critical daylength. In these plants, named long-day and short-day species, respectively, the duration of the critical daylength varies between species and also between varieties of the same species adapted to different latitudes.(2) Varieties of the same species grown at different latitudes flower in response to different photoperiod lengths; for example, varieties of cocklebur found in Florida flower when the daylength reaches 14 hours, but further north in Michigan they require 16-hour long days.(3)

Around 15 years after Garner and Allard’s original description of photoperiodism, Büning(4) proposed that plants might use the same time-keeping mechanism that regulates daily rhythms in leaf movements to measure daylength and, thereby, control seasonal responses. General acceptance that the daily timer (the circadian clock) controls daylength responses came much later, however, and stemmed from experiments in which plants were exposed to cycles longer than the daily cycle of 24 hours (Ref. 5; reviewed in detail in Ref. 2). The most striking of these involve growing plants under long periods of darkness and then inducing or repressing flowering by transient exposure to light at different times within the dark period. This demonstrates that the effect of these light treatments on flowering varies dramatically depending on when they are given within a 24-hour cycle, and that peaks in sensitivity to the treatments occur in a 24-hour cycle. For example, growth of the short-day plant Chenopodium rubrum in continuous light prevents flowering and flowering is induced by exposure to one dark period of 72 hours. Disruption of the dark period, however, with a 4-minute flash of light prevents flowering if given 36 or 60 hours into the dark period, but not if given 20 or 44 hours into it.(6) The 24-hour periodicity of the effect of light suggests that the light flashes prevent flowering by interacting with an underlying circadian rhythm. Similar results have been observed using a range of plant species with different responses to daylength, as well as in insects and birds. Furthermore, Arabidopsis mutants were described recently in which both circadian rhythms and flowering time control are disrupted and it is now generally accepted that the
circadian clock provides the time-keeping mechanism for photoperiodic responses (reviewed in Ref. 2).

In plants, temperature is also important in controlling seasonal responses. Perhaps the most dramatic of these effects is the initiation of developmental events in response to extended exposures to low temperatures. In this response, termed vernalisation, developmental processes such as the initiation of flowering are repressed until the plant has been exposed to an extended period of low temperature similar to those experienced in winter. Flowering of many plant species that grow at high latitudes is repressed until they have been exposed to such conditions, and this repression of flowering occurs even if the plant is growing under photoperiods that would otherwise promote flowering. For example, although exposure to long days (LDs) promotes early flowering in many varieties of *A. thaliana*, varieties that require low temperature treatments will not flower early even in LD conditions unless they have been previously exposed to low temperatures. The most successful treatments require exposure to low temperatures for a few weeks, suggesting that vernalisation ensures that flowering occurs in the spring following exposure to winter conditions. Thus, flowering in the spring or early summer occurs through a combination of exposure to the preceding winter, causing vernalisation, plus longer photoperiods that would otherwise promote flowering.

Finally, the age of a plant can determine whether it will flower in response to environmental stimuli. For example, the life cycle of many plants includes a juvenile phase during which they will not flower even in environmental conditions that would induce flowering in older plants. The duration of this juvenile phase varies dramatically between species and, in trees, it may last many years. Transition from the juvenile to adult phase can often also be observed in the organs that form at different stages during vegetative development and, in some species, these changes are related to the acquisition of the ability to flower. For example, in *Arabidopsis*, mutations that accelerate the transition between phases of vegetative development and cause early flowering have been identified. In contrast, in maize, mutations that extend the juvenile phase of vegetative development do not affect the age at which the plants can be induced to flower. These phases demonstrate that there is a mechanism for generating age-related changes in the development of the plant shoot that can also influence flowering time. These developmental phases are outside of the scope of this article, but have been reviewed recently.

In this article, we mainly review recent progress in understanding the response to daylength and its role in the control of flowering time. We also briefly describe recent advances in understanding vernalisation. Much of the recent progress has come from molecular genetic approaches in *Arabidopsis*, and we will largely concentrate on these experiments and on the role of the genes that they have identified. More general reviews describing the control of flowering time have appeared recently.

### Vernalisation: the promotion of flowering by extended exposures to low temperature

Vernalisation is most effective when plants are exposed to low temperatures for prolonged periods lasting several weeks. For example, exposure of *Arabidopsis* seedlings to 4°C for 6 weeks results in a maximal response, while treatment for 2 weeks gives a much lesser effect. This response can be considered a form of biological timer that ensures that flowering is repressed until the end of the winter months.

Vernalisation has been analysed genetically in *Arabidopsis* by comparing naturally occurring varieties that either do or do not respond to low temperature treatments. In varieties that do respond, exposure to low temperatures causes the plants to flower much earlier than they would if not exposed. These two response types differ at two major loci, *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*), which act together to delay flowering. The *FLC* gene was cloned recently and encodes a MADS box transcription factor that represses flowering when expressed at high levels (Fig. 1). Low temperature treatment of plants for 3 weeks, however, reduces the expression of *FLC*, and this correlates with early flowering. This suggests that during vernalisation low temperature treatments reduce the transcription of genes that repress flowering such as *FLC*. Similarly, expression of *FRI* or mutations in the *FCA* gene increases *FLC* levels and so delays flowering unless the plant is exposed to low temperatures (Fig. 1). The kinetics with which *FLC* expression is reduced during low temperature treatments is not known, and it will be interesting to assess whether increasing cold exposure from a few days to 3 weeks causes progressively lower levels of *FLC* expression. This is suggested by analysis of *FLC* mutant alleles that are expressed at higher levels than wild-type, which in turn repress flowering more dramatically. Plants with these mutant *FLC* alleles require longer low temperature treatments to flower early, as exposure to low temperature does not reduce *FLC* as rapidly as wild-type and this correlates with an incomplete effect of vernalisation on the acceleration of flowering.

How is transcription of *FLC* progressively reduced in response to exposure to low temperatures over a period of several weeks? One possibility is that progressive changes in gene methylation during the course of vernalisation change the pattern of *FLC* expression, and perhaps also the expression of other flowering time genes. Evidence supporting this comes from the observation that in some plants reduction of methyl transferase activity causes early flowering. These plants also have reduced expression of
Genetic screens have also been performed for mutations that prevent vernalisation, and at least one of the mutations identified, vernalisation 2 (vm2), blocks the decrease in FLC expression that normally occurs during low temperature treatment. Therefore, the processes required for vernalisation should be amenable to genetic analysis.

**Daylength measurement involves the circadian clock**

A mechanism by which plants measure the duration of a photoperiod is a prerequisite for the photoperiodic control of flowering time. The circadian clock that controls daily rhythms in gene expression and behaviour has been proposed to act as the timer in photoperiodic response as described above. Circadian rhythms have been widely studied in insects, fungi, plants and mammals (Fig. 2). Features of these rhythms are that the duration of one cycle is approximately 24 hours, that they are entrained to (or synchronised with) the day/night cycle by environmental changes in light/dark or in temperature, and that the rhythm persists when organisms are shifted from light/dark cycles to continuous conditions of light or dark. The circadian clock that controls these rhythms is often considered in three parts: a central oscillator that creates the 24-hour periodicity, input pathways to the oscillator that synchronise the oscillation to the day/night cycle, and outputs from the oscillator that are overt rhythms in gene expression and behaviour. Figure 2 illustrates these processes schematically in relation to the Arabidopsis circadian clock and they are discussed in more detail in following sections.

In plants, entrainment of the clock to daily cycles of light and dark is controlled by light receptors, including the phytochromes (red/far-red light receptors) and cryptochromes (blue light receptors). These will be discussed in detail later. The proteins that form the central oscillator of the plant circadian clock are unknown, although candidate genes and proteins are discussed in the next section. In other systems, such as Drosophila, Neurospora, and mammals, the proteins required for the central oscillator form a negative feedback loop based on the control of transcription and translation of central clock molecules. For example, in Drosophila the proteins dCLOCK and CYCLE activate expression of the Period (Per) gene. As PER protein accumulates in the cytoplasm it heterodimerises with TIMELESS (TIM); when this heterodimer reaches a threshold concentration it is imported into the nucleus where it prevents the activation of Per by dCLOCK/CYCLE (reviewed in Ref. 22). This cycle of negative feedback control of PER on its own expression takes approximately 24 hours, and this cycle time is largely due to the time taken for the TIM/PER heterodimer to accumulate before it is imported into the nucleus. In Figure 2 it is assumed that the plant circadian clock is also based on such a negative feedback loop, and candidate proteins for oscillator components are described in the following section.

Output pathways in Arabidopsis control expression of a wide range of clock-controlled genes that peak in expression in different phases of the cycle, such as COLD AND CIRCADIAN REGULATED 2, GIANTEA (G), CHLOROPHYLL A/B BINDING PROTEIN 2, and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1). These pathways also control overt rhythms in leaf movement, stomatal opening, and hypocotyl elongation and, in addition, the photoperiodic control of flowering is also likely to represent an output pathway. How these output pathways are controlled by the oscillator is unknown but, because components of the central oscillator are themselves transcription factors (at least in Drosophila), they may directly activate genes involved in particular output pathways.

**Genes that alter or disrupt circadian regulation in Arabidopsis**

In Arabidopsis, three mutations and one transgene that disrupt or alter normal circadian output have been described. Three of the relevant genes have been recently cloned and two of these, LATE ELONGATED HYPOCOTYL (LHY) and CCA1, encode very similar proteins that contain a single...
MYB repeat which suggests that these proteins bind DNA and regulate transcription. Indeed, the CCA1 protein does bind to specific DNA sequences. Expression of both LHY and CCA1 mRNAs have a diurnal rhythm in plants grown in light/dark cycles, with peak levels occurring at dawn. A similar pattern of expression has been shown for the CCA1 protein. When moved to constant light conditions, plants express both genes in a circadian rhythm, and LHY also maintains a circadian rhythm of expression in constant darkness. Circadian patterns of LHY and CCA1 expression may be required to maintain all circadian outputs. Constant high levels of either transcript in transgenic plants disrupts all rhythmic outputs examined, both in constant light and in constant dark. Furthermore, overexpression of either gene causes a loss of rhythmicity in the expression of their own and each others transcript. 

Although these studies show that overexpression of LHY and CCA1 disrupts clock function, they do not prove that the genes are required for normal clock function. Recent characterisation of a CCA1 loss-of-function mutant, however, demonstrates that CCA1 is required for circadian clock regulation with a normal period. Entrained plants transferred to constant light showed a reduction of 3 hours in the period of expression of two clock-regulated genes that normally peak at the same time as CCA1. Genes that normally peak later in the day also had an abnormal expression pattern that might also be explained by a shorter period. Whether CCA1 is also required for normal rhythms in constant dark or in light/dark conditions has not yet been reported. The phenotype of the CCA1 mutant demonstrates that, although CCA1 and LHY are closely related in sequence, LHY cannot completely compensate for the loss of CCA1, although it may do so partially. Characterisation of a LHY loss-of-function mutant is in progress (K. Wheatley and G. Coupland, unpublished). At this time, attempting to place either LHY or CCA1 in the input, output, or within the oscillator itself is premature.

Constant overexpression of either LHY or CCA1 causes late flowering in long days, and LHY overexpression causes

Figure 2. A schematic model of circadian clock control in Arabidopsis. Light signals entrain the circadian clock to the daily cycle of night and day. Phytochrome and cryptochrome photoreceptors convey the “input” signals to the central oscillator. The oscillator generates the 24-hour rhythm, and is proposed to be a negative feedback loop with properties similar to those described in other systems. In this scheme the expression of clock genes is promoted by “positive elements.” As the abundance of clock gene products rises, they repress their own synthesis by antagonising the function of the “positive elements.” The abundance of clock gene products then falls and the function of the “positive elements” is restored. This regulation produces peaks in clock gene expression that are approximately 24 hours apart. The “positive elements” might also regulate the expression of clock-controlled genes that direct overt rhythms or output from the oscillator. In Arabidopsis, these include leaf movements, hypocotyl elongation, the expression of many genes and the photoperiodic control of flowering time.

Figure 3. Genetic and molecular interactions involved in photoperiod response in Arabidopsis. Four genes (CCA1, LHY, TOC1, and ELF3, shown in red) are proposed to act within the central oscillator of the circadian clock or to act at positions in input or output pathways that are closely associated with oscillator function. The oscillator is entrained to the day/night cycle by light receptors, and CRY1 and PHYB (shown in green) play an important role in this entrainment under high fluence light conditions. The phase of expression of genes regulated by the circadian clock differs in response to long or short daylengths, although not all of these genes have a role in flowering regulation. The phase of expression of flowering time genes that occurs under long days is proposed to trigger the promotion of flowering through the GI and CO genes. GI also affects the amplitude of LHY and CCA1 expression. CRY2 and PHYA photoreceptors promote flowering in response to photoperiod, and CRY2 increases the abundance of CO mRNA. Whether PHYA (shown in brackets) acts in a similar way is not known. The FT and FWA genes act downstream of CO.
the flowering time of plants to become insensitive to daylength. The correlation between the flowering-time phenotype and circadian clock function suggests that, in Arabidopsis, the response to photoperiod requires a functional circadian clock. The basis of this may be the regulation of expression of flowering-time genes which have reduced and abnormal diurnal patterns of expression in plants that over-express LHY (see below).

The phenotype of loss-of-function early flowering 3 (elf3) mutants suggest that ELF3 is required to maintain circadian output under certain conditions. Circadian output in Arabidopsis is often investigated using a fusion between the promoter region of the circadian clock-controlled gene CAB2 and the luciferase marker gene (CAB:LUC). elf3 mutant plants entrained in light/dark cycles and moved to continuous white light showed arrhythmic CAB:LUC expression, as well as arrhythmic leaf movements and LHY expression. On the other hand, when moved into constant dark loss of ELF3 had no significant effect on CAB:LUC rhythmicity. Plants entrained to different light/dark cycles suggest that CAB:LUC expression is normal in short days but becomes abnormal as the photoperiod lengthens. In addition, elf3 mutant plants grown in short-day (SD) conditions, there are significant changes in the expression of genes that normally peak later in the day than CAB:LUC (see below).

Another gene, TIMING OF CAB 1 (TOC1) is also thought to play an important role in circadian regulation. In continuous light, plants that express the semi-dominant toc1-1 mutant have a shorter period for several markers and, in some strains of Arabidopsis, this results in early flowering. The mutation probably causes the central oscillator to run at a higher frequency in the toc1-1 mutant plants in different light/dark cycles has not been described, but under a 24-hour temperature entrained cycle the abnormal waveform of CAB:LUC in plants indicates that TOC1 is required for correct processing of the entraining signals. This indicates that entrainment to a cycle (in this case a 24-hour temperature cycle) that differs greatly from the period of the endogenous clock (21 hours for toc1) results in a distortion in the normal rhythmic pattern of gene expression. Indeed, in Drosophila expression of different mutant alleles of PER causes short or long periods under free-running conditions and results in a shift in the timing of behavioural patterns and gene expression under light/dark cycles.

**Photoreceptors in Arabidopsis and their role in circadian clock entrainment**

Two types of light receptors have been analysed in detail in Arabidopsis. The first of these, cryptochromes, are flavoproteins that function as blue light receptors. CRYPTOCHROME 1 (CRY1) encodes a soluble protein that is expressed at similar levels in dark- and light-grown seedlings. Loss-of-function mutants show reduced sensitivity to blue light and transgenic gain-of-function mutants exhibit increased photosensitivity. CRYPTOCHROME 2 (CRY2, also known as FHA) encodes a soluble light-labile protein.

The second group of light receptors, phytochromes, are encoded by a small family of five genes in Arabidopsis (PHYA to PHYE). Each phytochrome exists in two forms. The Pr form is converted to the Pfr form by exposing plants to red light, and the reverse reaction occurs in far-red light. Physiological and mutational analyses have shown that PHYA and PHYB have distinct yet overlapping functions. The PHYA gene encodes a protein that is abundant in dark-grown seedlings and, whereas the Pr form of PHYA is stable in the cell, the Pfr form is subject to rapid degradation, which results in very low PHYA levels in red light. Analysis of phyA mutants has revealed that PHYA is the primary, if not the only, phytochrome responsible for de-etiolation in continuous far-red light. On the other hand PHYB encodes a light-stable protein that is the primary phytochrome responsible for de-etiolation in response to red light and is a major contributor to the shade avoidance response.

The effects of the cryptochromes and phytochromes on entrainment of the circadian clock have been investigated by analysing the effect of mutations on CAB:LUC expression, and mutations in genes encoding CRY1, CRY2, PHYA, and PHYB influence circadian clock-entrainment under specific conditions. However, under conditions of high light intensity (high fluence) CRY1 and PHYB are the most important light receptors in circadian clock entrainment. Mutations in CRY1 caused a lengthening in period under high fluence red light, while mutation in phyB has similar effects under high fluence red light. Although mutations in CRY2 and PHYA had no effect under high fluence conditions, phyA lengthened period under low fluence red or blue light and phyB caused a slight shortening of period under low fluence blue light and had no effect on period length under white light. Clearly, therefore, there is redundancy between light receptors that entrain the plant circadian clock. It is striking, however, that phyA and cry2, which have marked effects on the photoperiodic control of flowering (see below), only affect clock entrainment under specialised low fluence conditions. This suggests that these genes do not affect daylength responses by influencing circadian clock entrain-
ment but by modulating other aspects of the daylength response. (54)

It is not known how these effects relate to the expression of genes such as LHY and CCA1 proposed to be involved in the regulation of circadian rhythms. It is known, however, that CCA1 expression responds rapidly to exposure of etiolated seedlings to light, and that loss of function of both PHYA and PHYB causes an 8-hour delay in red light induction of the CRY gene. (47) If these loss-of-function mutations were to have similar effects on CCA1 and LHY expression, one could envisage a mechanism by which the phase of all circadian outputs could be altered. When plants are grown in the dark, CRY1 and PHYA proteins also modify gene expression of some circadian-regulated genes. For example, expression of the CAT3 gene increases and oscillations are rapidly lost when plants are moved to constant dark, and functional CRY1 and PHYA proteins are required for this response. (55) Recently, evidence of physical interactions between CRY1 and PHYA proteins has been reported, (56) which suggests that CRY1 and PHYA might work together in the input of blue light in very low fluence rates and in regulation of gene expression of clock-regulated genes.

**Photoperiodic response in Arabidopsis and the role of photoreceptors**

Arabidopsis plants grown in LD conditions flower earlier and with fewer leaves than those grown under SDs. (57, 58) A response to LDs can be detected soon after germination; seedlings grown in LDs and shifted to SDs when they are 8–10 days old will flower at a similar time to plants grown continuously in LDs. (59) Furthermore, older Arabidopsis plants respond rapidly to longer daylengths and exposure to a single LD can be sufficient to induce flowering. (60–62) Short exposures to light in an otherwise noninductive long dark period can also, under certain conditions, promote flowering of Arabidopsis, and far-red and blue night breaks are the most efficient in eliciting this response. (58) Thomas and Vince-Prue (2) reported a circadian rhythm in the response to night break in Arabidopsis.

Photoreceptors which seem to be specifically required for promotion of flowering in Arabidopsis under LD conditions are CRY2 and PHYA. Loss-of-function CRY2 mutants have no flowering-time phenotype in SDs and are late-flowering in LDs, (59) although they still respond weakly to changes in photoperiod. (63) The flowering phenotype is more pronounced in some varieties (e.g., Columbia) than others (e.g., Landsberg erecta). A mutation in CRY2 delays flowering of plants grown in constant red, red-plus-blue or white light, (43) and transgenic gain-of-function mutants are early flowering in SDs. (43) As described earlier, cry2 does not affect circadian clock entrainment and probably alters flowering time by directly influencing the expression of flowering time genes. Although cry1 mutations affect flowering time, and most alleles cause late flowering in SDs, they do not affect flowering time in response to extended short days, night break treatments, (58, 66) nor constant blue light treatments, (43) suggesting that CRY1 is not involved in LD promotion of flowering.

The phyA-1 mutant is late-flowering in extended SD conditions and has a reduced response to night break treatment. (47, 67) Overexpression of PHYA causes early flowering in SDs as well as photoperiod insensitivity. (68) As with cry2, the effect of phyA on the response to photoperiod is probably not due to direct effect on circadian clock itself, but is more likely to be a result of an independent PHYA signalling pathway. (54) It is interesting that both PHYA and CRY2 are light-labile, the absence of either results in late flowering in LDs, and neither is required for normal period length of the circadian clock in high irradiance conditions. We propose a possible role for these receptors in modulating the expression or function of flowering time genes.

**Genes that affect flowering in response to photoperiod in Arabidopsis**

In addition to cry2 and lhy discussed above, mutations in other genes, constans (co), gigantea, (gi); ft; fwa, that disrupt photoperiodic responses have also been described (reviewed in Refs. 14, 16). These mutations delay flowering under LDs but have no, or only slight, effects under SDs, and are considered as defining genes required to promote flowering in response to LDs. (64, 68–74) As double mutants, carrying combinations of cry2, co, gi, or lhy, flower at approximately the same time as the later of the single mutants it has been proposed that they all function in the same pathway, called the long-day pathway (14, 71) (Schaffer, Wheatley, and Coupland, unpublished). The ft and fwa mutations have different genetic interactions compared to the other four genes in this pathway, for example, when ft and fwa are combined with mutations in the floral meristem identity gene LEAFY the double mutants almost completely lack floral tissues. (75)

This suggests that they may act in a different aspect of the photoperiodic response. CRY2 and LHY were described in earlier sections, and most of the other genes in this group have now been cloned. CO encodes a zinc-finger protein that is localised to the nucleus and is most likely a transcription factor (58) (Costa, Pino, and Coupland, unpublished). GI encodes a large protein with several possible membrane-spanning domains. (25) The FT gene encodes a putative phosphatidylethanolamine-binding and nucleotide-binding protein (76, 77) similar to TERMINAL FLOWER (TFL), (78) although the mode of action of these proteins is still unknown. FWA is a gain-of-function mutant, and the gene has not yet been cloned.

The order in which these genes act within the pathway has been analysed at the molecular level by comparing their
expression in mutant and wild-type plants, and by creating transgenic plants which overexpress individual genes in wild-type and mutant backgrounds. Transgenic plants in which CO is overexpressed from the 35S viral promoter flower very early and the plants are insensitive to daylength.(79) This gain-of-function CO transgene is epistatic to gi, cry2, and lhy mutations but ft and fwa delay flowering of 35S:CO (Igeno, Robson, Onouchi, Wheatley, Coupland, unpublished). These genetic interactions suggest that CO acts downstream of GI, CRY2, and LHY, but upstream or parallel to FT and FWA in the LD promotion pathway. Similarly, expression of CO is reduced in late-flowering cry2 mutants and increased in early-flowering plants that overexpress CRY2, suggesting that CO acts downstream of CRY2. A loss-of-function mutation in ELF3 and gain-of-function mutations in LHY and CCA1 affect expression of GI transcript, as expected for a circadian-regulated gene.(25) The reduction in LHY and CCA1 expression seen in gi mutants, however, suggests that these genes do not act in a straightforward linear way, but that they influence each other’s expression.(25) The expression patterns of several of the genes in this pathway are regulated by the circadian clock and are altered by daylength, suggesting that transcriptional regulation may activate this pathway under LD conditions. For example, at certain times of the daily cycle CO expression is higher in long than short days(80) (Suarez-Lopez and Coupland, unpublished). The recent analysis of GI expression also suggests how changes in photoperiod might influence flowering time. Levels of the GI transcript are regulated by the circadian clock with peak expression occurring 8–12 hours after dawn.(25) The timing and duration of this peak is influenced by daylength: it is broader and extends into the night in longer days. It is possible, therefore, that alterations in the precise timing or structure of the circadian peak in expression may explain how this pathway is activated by daylength (also see following section).

A working model for time measurement in the control of flowering in response to changes in photoperiod

Two general models have been proposed to explain how daylength is measured in the control of developmental responses, such as flowering (reviewed in Ref. 2). One of these models, often called the external coincidence model, proposes that an underlying circadian rhythm is sensitive to light at particular phases of the rhythm, and if the plant is exposed to light at that time flowering will be either promoted in a long-day plant or repressed in a short-day plant. The second model, the internal coincidence model, proposes that two underlying rhythms are out of phase under conditions that do not induce flowering but are brought into phase under conditions that are inductive.

Recent molecular data have supported the potential for these types of models. As described earlier, genes that are required for the photoperiodic response of Arabidopsis are circadian-regulated and their phase of expression changes in LDs compared to SDs. An effect of photoperiod on the phase of overt rhythms was originally described for the eclosion rhythm in Drosophila(80) and has been demonstrated for the pattern of expression of the circadian clock-regulated gene CAB2.(81) Furthermore, regulation of flowering time by the phase of expression of flowering time genes may explain why mutations such as toc1 that alter the phase of expression of circadian clock-controlled genes are also altered in flowering time.(38)

Altersations in the temporal pattern of expression in response to photoperiod indicate that a model similar to the internal coincidence model might operate. For example, two rhythms regulated by the circadian clock may cycle in similar but distinct phases and overlap in expression under some, but not other, photoperiods. These rhythms could be in two flowering time genes, such as GI and CO, or in one flowering time gene product and a modulator of protein activity. One candidate for such a modulator is the level of free calcium in the cytosol. There is a circadian rhythm in cytosolic calcium concentration in Arabidopsis, with a peak occurring shortly after dawn.(82) Oscillations of free calcium within the range reported will affect the function of many cellular systems, and possibly flowering, and a time-dependent effect of calcium on flowering has been proposed in photoperiodic induction in the SD plant Pharbitis nil.(83)

Alternatively, the alterations in the rhythmic expression patterns detected under long and short days also provide support for the external coincidence model. For example, the phase of expression of flowering time genes might be altered by the light in long but not short days, and it is possible that light is needed to activate the proteins or their target genes. In this scenario, the light receptors PHYA and CRY2 are likely candidates to mediate this interaction, since both are required for LD perception. Both proteins are light labile, which suggests that they might act in dark or at dawn.

Conclusions

Genetic approaches in Arabidopsis have provided access to genes whose products control flowering in response to seasonal changes in temperature and daylength. The analysis of these genes has emphasised the role of the circadian clock in regulating the response to daylength. For example, the lhy and elf3 mutations or the overexpression of CCA1 disrupt circadian clock control and the photoperiodic response, while the expression of the GI gene, which promotes flowering in response to LDs, is regulated by the circadian clock. This has led to the formulation of models whereby the pho-
The periodic response is controlled by modulating the expression of flowering time genes in response to both the circadian clock and daylength. The genes that have been identified so far provide a general outline of the processes that regulate the photoperiodic response, but major questions remain. For example, we propose that inductive photoperiods promote flowering by changing the phase of expression of flowering time genes (Figs. 3, 4). This model is supported by the effects of different photoperiods on the pattern of expression of GI, but direct experimental evidence that this influences flowering time is required. This may also lead to the identification of factors that activate flowering time gene proteins in one phase of expression but not in another. The potential role of CRY2 and PHYA in this area is intriguing, since they have an effect on the photoperiodic response but not circadian clock entrainment. Furthermore, the analysis of flowering time mutants has identified genes that are important in the generation of circadian rhythms. Whether these genes act within the central oscillator is not clear but can be tested using approaches similar to those used in other systems.

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