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Modulation of environmental responses of plants by circadian clocks

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ABSTRACT

Circadian clocks are signalling networks that enhance an organism's relationship with the rhythmic environment. The plant circadian clock modulates a wide range of physiological and biochemical events, such as stomatal and organ movements, photosynthesis and induction of flowering. Environmental signals regulate the phase and period of the plant circadian clock, which results in an approximate synchronization of clock outputs with external events. One of the consequences of circadian control is that stimuli of the same strength applied at different times of the day can result in responses of different intensities. This is known as 'gating'. Gating of a signal may allow plants to better process and react to the wide range and intensities of environmental signals to which they are constantly subjected. Light signalling, stomatal movements and low-temperature responses are examples of signalling pathways that are gated by the circadian clock. In this review, we describe the many levels at which the circadian clock interacts with responses to the environment. We discuss how environmental rhythms of temperature and light intensity entrain the circadian clock, how photoperiodism may be regulated by the relationship between environmental rhythms and the phasing of clock outputs, and how gating modulates the sensitivity of the clock and other responses to environmental and physiological signals. Finally, we describe evidence that the circadian clock can increase plant fitness.

Key-words: abscisic acid; *Arabidopsis*; circadian gating; cold; light; signalling; stomata.

INTRODUCTION

In a world characterized by rhythms of light intensity and temperature cycles, there has been selection for the evolution of an internal clock that optimizes the plant's relationship with the environment. This circadian clock runs with a period close to 24 h, even in the absence of environmental cues, and maintains a relatively constant period within a physiologically relevant temperature range. The plant circadian clock can be considered to be a signalling network that carries time-encoded information and regulates physiological processes. In this review, we show how plants' interactions with the environment are influenced by the circadian clock. We describe how changes in the environment regulate the circadian clock, how circadian clock outputs optimize the plant's responses to the environment and how the circadian clock may improve plant fitness.

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STRUCTURE OF *ARABIDOPSIS* CIRCADIAN CLOCKS

Circadian clocks are usually thought of as being divided into three parts: a central oscillator, which generates the rhythmic behaviour; the input pathways, which carry environmental information to entrain the central oscillator; and the output pathways that regulate physiological processes (Fig. 1; Dunlap 1999). This conceptual model is very useful but overly simplistic as there is crosstalk between different parts of the circadian clock, most strikingly between the output and input pathways, with some circadian-regulated outputs modulating input into the oscillator (for reviews, see, Más 2005; Gardner *et al*. 2006; McClung 2006).

Here we use the term circadian clock to describe the whole circadian system. In plants, this system is likely to consist of more than one clock. There is good evidence for independent oscillators in each cell. It is also possible that there are cell-specific oscillators and multiple oscillators in individual cells (see Gardner *et al*. 2006, for a review).

Generation of circadian rhythms

The circadian clock generates rhythms that maintain a robust near-24 h period through a network of multiple feedback loops (Fig. 1). The first model for a plant circadian clock was a single-loop model composed of TIMING OF CHLOROPHYLL A/B BINDING PROTEIN 1/PSEUDO-RESPONSE REGULATOR 1 (TOC1/PRR1), CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY; Alabadí *et al*. 2001). *TOC1*, which is expressed with a peak 12 h after dawn (*zeitgeiber* time 12 or ZT12), is a member of a family of five pseudo-response regulators (TOC1/PRR1, PRR3, PRR5, PRR7 and PRR9; Millar *et al*. 1995a; Matsushika *et al*. 2000; Strayer *et al*. 2000). *CCA1* and *LHY* encode light-induced MYB-like transcription factors that are expressed highly during the early morning (ZT0, Schaffer

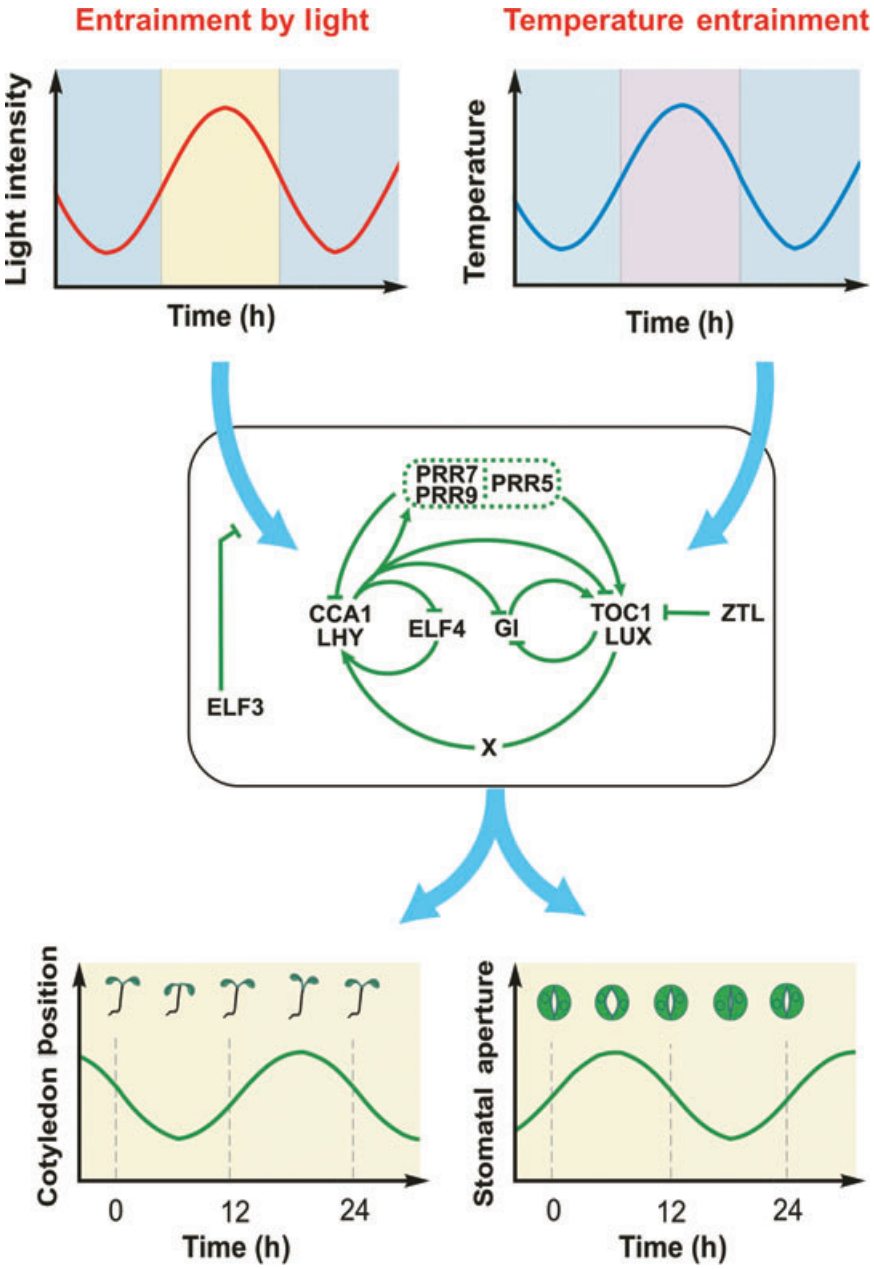

Figure 1. The generation of circadian rhythms in *Arabidopsis*. Environmental rhythms in light intensity and/or ambient temperature entrain the core circadian oscillator. The oscillator regulates a range of physiological outputs and maintains these rhythms in an appropriate phase relationship with the entraining environmental cues. A simplified model of the core oscillator, based on that proposed by Gardner *et al.* (2006), is illustrated. Arrows indicate a positive (inductive) relationship between components, and bars a negative (repressive) relationship. Component X is predicated from mathematical modelling (see text for details). Rhythms in cotyledon movement and stomatal opening are illustrated as examples of differently phased circadian outputs. CCA1, CIRCADIAN CLOCK-ASSOCIATED 1; ELF 3, EARLY-FLOWERING 3; ELF4, EARLY-FLOWERING 4; GI, GIGANTEA; LHY, LATE ELONGATED HYPOCOTYL; LUX, LUX ARRHYTHMO; PRR5, PSEUDO-RESPONSE REGULATOR 5; PRR7, PSEUDO-RESPONSE REGULATOR 7; PRR9, PSEUDO-RESPONSE REGULATOR 9; TOC1, TIMING OF CHLOROPHYLL A/B BINDING PROTEIN 1; ZTL, ZEITLUPE.

et al. 1998; Wang & Tobin 1998). In the single-loop model, light activates expression of *CCA1*/*LHY*, which represses the expression of *TOC1*. The decrease of TOC1 levels reduces the level of *CCA1*/*LHY* expression. At the end of the subjective day, CCA1/LHY levels have fallen sufficiently to allow *TOC1* to be expressed (Alabadí *et al.* 2001). CCA1/LHY binds to a motif called EVENING ELEMENT (EE, AAATATCT) in the *TOC1* promoter, repressing *TOC1* expression (Harmer *et al.* 2000; Alabadí *et al.* 2001). Overexpression of either *CCA1* or *LHY* (*CCA1*-ox and *LHY*-ox) leads to arrhythmia, with *TOC1* expressed at low levels (Alabadí *et al.* 2001). In turn, TOC1 indirectly induces the expression of *CCA1*/*LHY*, as the recessive loss-of-function *toc1*-2 mutant has reduced expression of *CCA1*/*LHY* (Alabadí *et al.* 2001).

A number of biological and mathematical studies have demonstrated that the single-loop model requires modification to explain fully circadian behaviour. The *cca1*-11 *lhy*-21 double mutant, for example, is a short-period mutant rather than being arrhythmic as the single-loop model predicts (Alabadí *et al.* 2002; Mizoguchi *et al.* 2002; Locke *et al.* 2005b). There is also a requirement for a delay mechanism between TOC1 and CCA1/LHY, because the translation peak of *TOC1* does not match the time that the transcription of *CCA1/LHY* begins (Alabadí *et al.* 2001; Locke, Millar & Turner 2005a). This is compelling evidence for the existence of additional components in the circadian clock. Recently, Locke *et al.* (2005b) used mathematical modelling to propose extensions to the single-loop model. The Locke model proposes the existence of

two extra hypothetical components: X and Y. X is a proposed delay mechanism between TOC1 and CCA1/LHY, and Y is suggested to regulate *TOC1* expression levels and, in turn, is regulated by light, CCA1/LHY and TOC1 (Locke *et al*. 2005b). The Locke model predicts that Y has one rapid light-induced peak of expression after dawn, which is repressed by CCA1/LHY, and a second circadian-controlled peak in the late afternoon. Data from high-resolution sampling experiments revealed that the expression pattern of *GIGANTEA* (*GI*) closely matched the predicted characteristics of Y (Locke *et al*. 2005b; Mizoguchi *et al*. 2005). Experimental studies have provided further evidence that GI is a necessary component of the central oscillator, the details of which are discussed later ('Temperature entrainment and compensation'). Recently, two different groups have independently derived a three-loop model from the Locke model. This three-loop model incorporates a negative feedback loop between *CCA1*/*LHY* and *PRR7*/*PRR9* (Locke *et al*. 2006; Zeilinger *et al*. 2006; see also Fig. 1).

Expression of *LUX ARRHYTHMO* (*LUX* or *PHYTOCLOCK1*, *PCL1*), which has a MYB DNA-binding domain, correlates with *TOC1* expression (Hazen *et al*. 2005; Onai & Ishiura 2005). Plants that overexpress *LUX* become gradually arrhythmic under constant light (LL) and constant dark (DD), whereas the loss-of-function *lux*-1 is arrhythmic in both conditions. This suggests that LUX has a central role in the generation of rhythms (Hazen *et al*. 2005; Onai & Ishiura 2005). *EARLY-FLOWERING 4* (*ELF4*), which is also expressed in the same phase as *TOC1*, might form another feedback loop in the clock. Light-induced expression of *ELF4* requires CCA1/LHY, while light-induced *CCA1*/*LHY* expression requires ELF4 (Kikis, Khanna & Quail 2005). Mutants of *ELF4* have varying period length before reaching arrhythmia after 24 h in LL (Doyle *et al*. 2002).

In addition to *TOC1*, there are at least four other members of the PRR family that are rhythmically expressed (for a review, see Mizuno & Nakamichi 2005). It has been proposed that PRR5, PRR7 and PRR9 are involved in light and temperature input to the clock (Farré *et al*. 2005; Nakamichi *et al*. 2005b; Salomé & McClung 2005a). PRRs have the structure of a classical response regulator but lack the conserved aspartate that is phosphorylated by a kinase in the two-component signalling pathway (Hwang, Chen & Sheen 2002). Whereas the *toc1*-2 mutant is arrhythmic under constant red light and DD (Más *et al*. 2003a), loss of function of the other *PRR*s leads to either a small period reduction (*prr3*-1 and *prr5*-3) or a small period extension (*prr7*-3 or *prr9*-1; see Nakamichi *et al*. 2005b for a summary; Michael *et al*. 2003b; Mizuno & Nakamichi 2005; Salomé & McClung 2005a). This suggests that there is degree of redundancy between these components. The *prr5*-11 *prr7*-3 double mutant has a circadian period that is 4 h shorter than wild-type plants (Nakamichi *et al*. 2005a,b), while the prr7-3 prr9-1 double mutant has up to a 6 h period extension (Farré *et al*. 2005; Nakamichi *et al*. 2005b; Salomé & McClung 2005a). Finally, the *prr5*-11 *prr7*-3 *prr9*-1 triple mutant is arrhythmic in both LL and DD (Nakamichi *et al*. 2005b).

Many other components may have a role in the circadian pathway. These include ARABIDOPSIS RESPONSE REGULATORS 3 and 4 (ARR3 and 4; Salomé *et al*. 2006), TIME FOR COFFEE (TIC, Hall *et al*. 2003) and a poly(ADP-ribose) glycohydrolase (TEJ, Panda, Poirier & Kay 2002). However, the exact mechanisms of their action, their positions in the pathway and relative importance are still unknown.

Environmental inputs to the circadian clock

The intrinsic period of the circadian clock of *Arabidopsis* varies between 22 h and 29 h depending on accession and growth conditions (Michael *et al*. 2003b). To be synchronized with environmental rhythms, the plant circadian clock has a series of mechanisms that feed environmental information into the oscillator, which adjusts its phase and maintains the circadian clock period close to 24 h (Fig. 1, for detailed review Millar 2004; Salomé & McClung 2005b; Somers 2005). As an example, *CHLOROPHYLL A/B BINDING PROTEIN 2* (*CAB2*), which encodes a protein necessary for the light-harvesting complex, has a peak of transcription that changes under different photoperiods but is usually close to the middle of the light period (Millar & Kay 1996). The synchronization between the circadian clock and the 24 h environmental period is mediated by a resetting mechanism that shifts the phase of the clock every cycle in response to environmental cues (Fig. 2). However, precise control of period is also necessary to maintain correct phase of circadian outputs with the environment. For example, the short period mutant *toc1*-1 can entrain to a 24 h period under 24 °C/20 °C thermocycles but the peak of *CAB2* expression occurs earlier in the day than in wild-type plants (Somers *et al*. 1998b). A similar mismatch is found in the long period mutant *ztl*-1 (*zeitlupe*-1), in which *GLYCINE-RICH RNA-BINDING PROTEIN 7/COLD, CIRCADIAN RHYTHM AND RNA BINDING 2* (*GRP7*/*CCR2*) transcription peaks later under light/dark (LD) cycles than in the wild type (Somers *et al*. 2000).

Light input pathways

Light affects both the phase and period of the circadian clock. A light pulse provided to plants in DD can change the phase of endogenous rhythms by varying degrees that are dependent on the light signal length and intensity. The time of day that the light signal is given determines whether the phase will advance or delay (Fig. 2a; Millar 2004). The period of the circadian clock under LL decreases as the intensity of light input to the oscillator increases. This is known as Aschoff's rule (Aschoff 1979, cited in Devlin & Kay 2000). Phase adjustment by light, which is dependent on the time of light treatment, provides evidence that the oscillator is correctly responsive to specific cues at appropriate times of day, while Aschoff's rule provides evidence for a continuous readjustment of phase that results in an altered period under LL (Somers 2005).

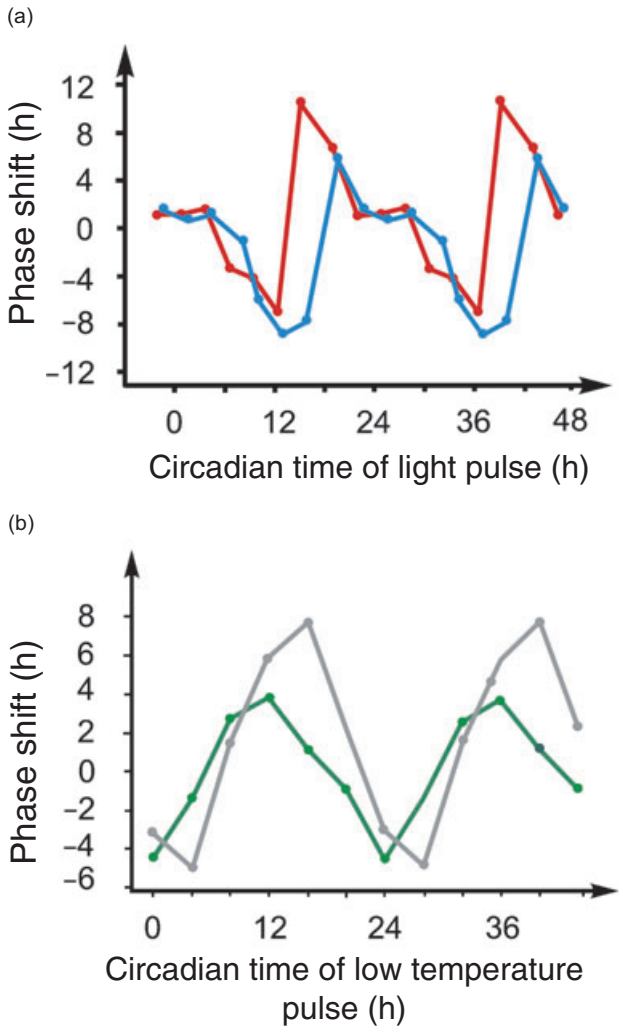

Figure 2. Gating of light and temperature input to the circadian clock. The phase of the circadian clock is set by light or temperature pulses. (a) The clock is most sensitive to red and blue light at and shortly after subjective dusk. Light pulses during the subjective day tend to delay the phase of the circadian clock, while pulses given during the latter part of the subjective night tend to advance the phase of the circadian pacemaker. The effect of light on the circadian clock was monitored using *COLD, CIRCADIAN RHYTHM AND RNA BINDING 2::LUCIFERASE* (*CCR2::LUC*) luminescence assays. Graphs redrawn from Covington *et al*. 2001 (© 2001 American Society for Plant Biologists, reprinted with permission). (b) Phase-response curves illustrating the effect of temperature pulses on the circadian clock. Seedlings grown in LD at 22 °C were released into LL received a 12 °C temperature pulse. The circadian clock is most sensitive to low temperature pulses at subjective dawn and subjective dusk, though the exact timing of the phase advance and delays depends on whether *TIMING OF CHLOROPHYLL A/B BINDING PROTEIN 1::LUC* (*TOC1::LUC*, green) or *CATALASE3::LUC* (*CAT3::LUC*, grey) is used to monitor the activity of the oscillator. Graphs redrawn from Michael *et al*. 2003b (© 2003 National Academy of Science, USA).

The main photoreceptors responsible for light input into the clock are the five red-light-sensing phytochromes (PHYA-E) and the two blue-light-sensing cryptochromes (CRY1 and CRY2; Somers, Devlin & Kay 1998a; Devlin & Kay 2000). The complexity of the input pathways to the clock is increased by some of their components being circadian-regulated (Harmer *et al*. 2000). For example, *CRY1* and *PHYB* expression peak in the middle of the day (ZT6), whereas *PHYA* and *CRY*2 expression peak in the latter part of the subjective day (ZT10; Tóth *et al*. 2001).

The role of PHY and CRY in the circadian clock was established by examining *phy*- and *cry*-null mutants under a wide range of light flux. PHYA has a role in setting the pace of the clock under red and blue low light flux, whereas PHYB has a role under high fluence red light (Somers *et al*. 1998a; Devlin & Kay 2000). The *phyA*-201 *phyB*-1 double mutant has longer periods than the wild-type plants in all fluence rates of red light and low fluences of blue light. Similarly, *cry1 cry2* (*hy4 cry2*-1) double mutants have longer periods than the wild-type plants in all fluences of blue light and low fluences of red light (Somers *et al*. 1998a; Devlin & Kay 2000). The less abundant PHYD and PHYE only appear to have a role when *PHYB* is mutated (Devlin, Patel & Whitelam 1998; Devlin *et al*. 1999). *PHY* mutants have a normal clock function under DD, which excludes a direct role of these components in the oscillator (Devlin & Kay 2000). The effects of *PHY* mutations under blue light and *CRY* mutations under red light indicate that there is crosstalk between the signalling pathways (Devlin & Kay 2000). There is no evidence that phototropins (PHOT), which mediate blue light responses in phototropism and stomatal movements are part of the circadian input pathway (Salomé & McClung 2005b).

Although the role of the photoreceptors in the clock is well described, little is known about the transduction of the light signal to the oscillator. Light has many putative entry points to the oscillator as transcription of *CCA1/LHY*, *GI*, *ELF4* and *PRR9* are induced by light (Wang & Tobin 1998; Martínez-Garcia, Huq & Quail 2000; Kim *et al*. 2003; Farré *et al*. 2005; Kikis *et al*. 2005; Locke *et al*. 2005b). One component believed to link light signalling and the oscillator was PHYTOCHROME-INTERACTING FACTOR 3 (PIF3), a transcription factor that interacts with both PHYA and PHYB (Ni, Tepperman & Quail 1998, 1999). PIF3 has been shown to form a complex with the G-box element of *CCA1/LHY* and the far-red (active) form of PHY (Pfr; Martínez-Garcia *et al*., 2000). Furthermore, red light induction of *CCA1*/*LHY* is attenuated in some *PIF3*-antisense lines (Martínez-Garcia *et al*. 2000). These data suggest that PIF3 is involved in the light-regulation of *CCA1*/*LHY* and, consequently, the oscillator. In addition, PIF3 interacts with TOC1 in yeast two-hybrid assays (Yamashino *et al*. 2003). However, the manipulation of *PIF3* levels (*PIF3* overexpression, *PIF3* antisense and *pif3* null lines) had no effect on the period or phase of the clock, which suggests that PIF3 does not have an important role in the regulation of the plant circadian clock (Monte *et al*. 2004; Oda *et al*. 2004; Viczián *et al*. 2005).

Light may also signal to the oscillator through DE-ETIOLATED 1 (DET1), a negative regulator of PHY and CRY signalling. The *det1*-1 mutant has a reduced

circadian period, possibly through the inhibition of LHY degradation (Millar *et al*. 1995b; Song & Carré 2005). CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) is believed to function in a similar light transduction pathway as DET1. The mutant *cop1*-6 reduces the period of the clock (Millar *et al*. 1995b; Ma, Zhao & Deng 2003; Song & Carré 2005). *FAR-RED ELONGATED HYPOCOTYL 1* (*FHY1*) and *FHY3* are two genes related to PHYA signalling that are required for phase shifting of leaf movement rhythms in response to far-red light (Yanovsky *et al*. 2001). Furthermore, mutation of *SUPPRESSOR OF PHYA-105* (*SPA1*), which is also involved in PHYA signalling, leads to a small reduction in the free-running period of *TOC1* and *CCA1* expression (Ishikawa, Kiba & Chua 2006). Finally, mutation of *SENSITIVITY TO RED LIGHT REDUCED 1* (*SRR1*), which is related to PHYB signalling, also causes a shortening of the circadian period of leaf movement and *TOC1* and *CCA1* expression. *SRR1*, however, may act through a pathway independent of PHYB because the short-period phenotype is still observed under DD (Staiger *et al*. 2003).

Another route for light input mediated by PHY and CRY may be ZEITLUPE (ZTL). ZTL targets TOC1 for degradation in a light-dependent manner (Más *et al*. 2003b). ZTL is part of a protein family that contains three characteristic protein domains: a central F-box, a LOV (LIGHT, OXYGEN, VOLTAGE) domain, which is similar to the chromophore-binding domain of PHOT, and six C-terminal KELCH repeats (Nelson *et al*. 2000; Somers *et al*. 2000; Schultz *et al*. 2001). Furthermore, the LOV domain changes conformation when exposed to blue light *in vitro* (Imaizumi *et al*. 2003). However, all described mutant alleles of *ZTL* have a long-period phenotype not only in LL but in DD as well. This suggests that light might not be an important regulator of ZTL (Kevei *et al*. 2006). Another member of the ZTL family, LOV/KELCH PROTEIN 2 (LKP2), might regulate TOC1 degradation in a similar fashion. However, *lkp2* has no circadian phenotype, which suggests that either LKP2 is not associated with clock function or there is a degree of redundancy (Salomé & McClung 2005b; Somers 2005). Both *ZTL* and *LKP2* overexpression lead to arrhythmia, which could be caused by increased degradation of TOC1 (Schultz *et al*. 2001; Más *et al*. 2003b). A third member of the ZTL family, FLAVIN-BINDING KELCH REPEAT F-BOX 1 (FKF1) does not have a role in the clock but is important in the photoperiodic control of flowering (Imaizumi *et al*. 2003) and is discussed later ('Circadian clock and seasonal responses').

Temperature entrainment and compensation

The circadian clock is entrained by cycles of temperatures with amplitudes greater than 4 °C (Fig. 2b; Somers *et al*. 1998b; McWatters *et al*. 2000; Michael, Salomé & McClung 2003a; Hazen *et al*. 2005). It is proposed that PRRs are involved in temperature input to the clock because *prr7*-3 *prr9*-1 double mutants grown under a 22 °C/18 °C thermocycle are arrhythmic in constant environmental conditions. Similarly, the double mutant failed to entrain when transferred from LD cycles to 22 °C/18 °C thermocycles (Salomé & McClung 2005a,b).

As temperature affects the rate of biochemical reactions, one might expect temperature to affect the pace of the circadian clock. However, circadian period does not change significantly in response to a range of physiologically relevant temperatures (from 12 °C to 27 °C in *Arabidopsis*; Edwards *et al*., 2005). This demonstrates that there are mechanisms to compensate temperature-induced changes in reaction rates. Characterization of circadian clock gene expression at different temperatures showed that *LHY* expression decreased in high temperatures. This was counterbalanced by increases in *TOC1* and *GI* levels. In contrast, in low temperatures, expression levels of *CCA1* increased slightly, which was counterbalanced by small reductions of *GI* levels. In addition, the null mutant *gi*-11 and the loss-of-function mutants *cca1*-11 and *lhy*-21 did not compensate for temperature changes (Gould *et al*. 2006). These data suggest that temperature-induced changes in *GI*, *CCA1* and *LHY* levels compensate for temperature changes, resulting in maintenance of the period of the circadian clock. Distinct roles for *CCA1* and *LHY* in temperature compensation were established by studying the effects of low and high temperature treatments in loss-of-function lines. Changes in the period of the circadian clock in response to temperature were most severe in high temperatures for *lhy*-21 (27 °C), while the effects were greatest at low temperatures (12 °C; Gould *et al*. 2006) for *cca1*-11. The *FLOWERING LOCUS C* (*FLC*) null-mutant *flc*-3 has short periods in 27 °C but not in 22 °C or 15 °C, which suggests that *FLC* is required for period control specifically in warm temperatures (Edwards *et al*. 2006). The period-shortening phenotype was even more pronounced if *FRIGIDA* (*FRI*-SF2), a positive regulator of *FLC*, was also mutated. In 27 °C, the *FRI*-SF2 *flc*-3 double mutant had the same expression levels of *TOC1*, *CCA1*/*LHY* and *GI* as the wild type. However, *LUX* levels in the *FRI*-SF2 *flc*-3 double mutant were higher in 27 °C but not in 22 °C, which suggests that *FLC* and *FRI* have a role in temperature compensation through the repression of *LUX* in 27 °C (Edwards *et al*. 2006).

Outputs of the circadian clock and the environment

Little is known about how the temporal information in the circadian clock is transduced to bring about rhythms in physiological and biochemical events, but at least three regulatory mechanisms are likely to be involved: the control of the protein abundance, the regulation of enzymatic activity and alterations in metabolite levels. The circadian clock may regulate the abundance of proteins involved in metabolic pathways through the control of gene transcription, RNA stability, translation rates or regulation of protein degradation. Alternatively, regulatory proteins with rhythmic activity may modulate the activity of proteins of a

metabolic pathway. Finally, there might be rhythms in metabolites necessary for the metabolic pathway to proceed effectively (Harmer *et al*. 2000; Harmer, Panda & Kay 2001).

Approximately 2–16% of the genes expressed in *Arabidopsis* have circadian rhythms in the steady-state levels of transcript abundance (Harmer *et al*. 2000; Schaffer *et al*. 2001; Edwards *et al*. 2006). Among these rhythmically expressed transcripts, many encode proteins that are involved in a large number of the *Arabidopsis* metabolic pathways. Furthermore, many of the clock-regulated transcripts encode proteins with regulatory roles, such as kinases and phosphatases, so it is possible that the level of the circadian control over plant metabolism is even greater than suggested by the percentage of transcripts under circadian control (Harmer *et al*. 2000). Some mechanisms involved in the circadian control of transcript abundance have been determined. The EE confers evening-phased expression to genes (Harmer *et al*. 2000; Alabadí *et al*. 2001; Michael & McClung 2002). A skeleton promoter of four tandem repeats of the EE is sufficient to result in rhythmic evening-phased gene expression, probably because of rhythmic *CCA1*/*LHY* expression and binding to the promoter, repressing expression (Harmer & Kay 2005). It is also possible that EE acts to promote transcription during the morning (Harmer & Kay 2005). Another CCA1/LHY-binding promoter motif, CBS (AAAAATCT), confers dawn-phased rhythmic expression through the positive action of CCA1/LHY (Wang *et al*. 1997; Piechulla, Merforth & Rudolph 1998; Michael & McClung 2002).

There may be a link between the phase at which transcript abundance peaks and the function of its product. Some circadian-regulated genes, such as those involved in photosynthesis, flavonoid synthesis, cell elongation, nitrogen fixation and mineral assimilation, may have a direct impact on the response of the plant to the environmental rhythms (Harmer *et al*. 2000; Schaffer *et al*. 2001; Edwards *et al*. 2006). Transcripts encoding proteins involved in photosynthesis or required for the synthesis and binding of photosynthesis pigments tend to be expressed during the time of maximum light intensity (between ZT4 and ZT8, Harmer *et al*. 2000). Likewise, transcripts required for phenylpropanoid synthesis, whose products protect the plant against ultraviolet (UV) radiation (Landry, Chapple & Last 1995), peak before dawn (ZT20), possibly to prevent photodamage during the day (Harmer *et al*. 2000). Furthermore, the induction of stress-related genes in the late afternoon (ZT8) may anticipate water-deficit stress because of extended stomatal opening in the afternoon and cold in the early evening (Harmer *et al*. 2000; Kreps *et al*. 2002). Transcript abundance may oscillate not only to anticipate plant responses caused by rhythmic changes in the environment, as in the examples above, but also to rhythmically modulate the way in which a plant responds to environmental stimuli. In the following section, we discuss the rhythmic alterations in the plant's responses to the environment.

CIRCADIAN MODULATION OF ENVIRONMENTAL RESPONSES

One of the consequences of circadian control is that stimuli of equal strength applied at different times of the day can result in a different intensity of response (Fig. 3). This phenomenon is called gating. One example of gating is the diurnal variation in the inhibition of stem elongation by wind (Gaal & Erwin 2005). When wind perturbation was given to *Cosmos bipinnatus* at different times of the day, the most intense effect on growth was observed when wind was applied during the day (Gaal & Erwin 2005). Similarly, inhibition of stem growth in the legume *Phaseolus vulgaris* by mechanical stimulation was also greatest at the beginning of the day (Fig. 4a; Anderson-Bernadas *et al*. 1997). These data suggest that there is rhythmic sensitivity to mechanical stimuli in a range of plant species and that the circadian clock is a likely controller of rhythmic sensitivity to extracellular signals.

Gating of a signal may allow plants to better process and react to the wide range and intensities of environmental signals to which they are constantly subjected. The changes of sensitivity to environmental signals also may allow plants to respond only when it is advantageous. The circadian clock may gate a signalling pathway through a direct mechanism (Fig. 3a), in which outputs of the clock are part of the signalling pathway, or an indirect one, in which the clock regulates a specific gating pathway whose role is to modulate other signalling pathways (Fig. 3b) or both (Fig. 3c). In all cases, gating is a consequence of circadian control and can therefore operate via the regulation of the abundance of signalling intermediates, the control of the activity of signalling molecules or the availability of metabolites involved in the pathway (see 'Outputs of the circadian clock and the environment'; Harmer *et al*. 2000). As examples, PHY and CRY abundance are controlled by the clock, which may result in the gating of light input to the clock (Harmer *et al*. 2000; Tóth *et al*. 2001; Sharrock & Clack 2002). Alternatively, gating of light induction of *GI* expression is mediated by the binding of CCA1/LHY to the EE in its promoter (Locke *et al*. 2005b).

Gating of light responses

We have briefly described gating of light input to the circadian oscillator and of light-induced expression of *GI*. Light-induced transcription of *CAB2* and shade-avoidance responses are other examples of light signals gated by the circadian clock. The promoter of the *CAB2* gene is regulated by both light and the circadian clock. The intensity of the acute light-induced increase of *CAB2* expression is dependent on the time of the day the light stimulus is applied (Fig. 4c; Millar & Kay 1996). The highest response of *CAB2* expression to light in DD was observed around 30 h and, the lowest, 18 h and 42 h after transfer to DD, which coincide, respectively, with the maximal and minimal levels of *CAB2* transcription in DD (Millar & Kay 1996; McWatters *et al*. 2000). In null mutants of

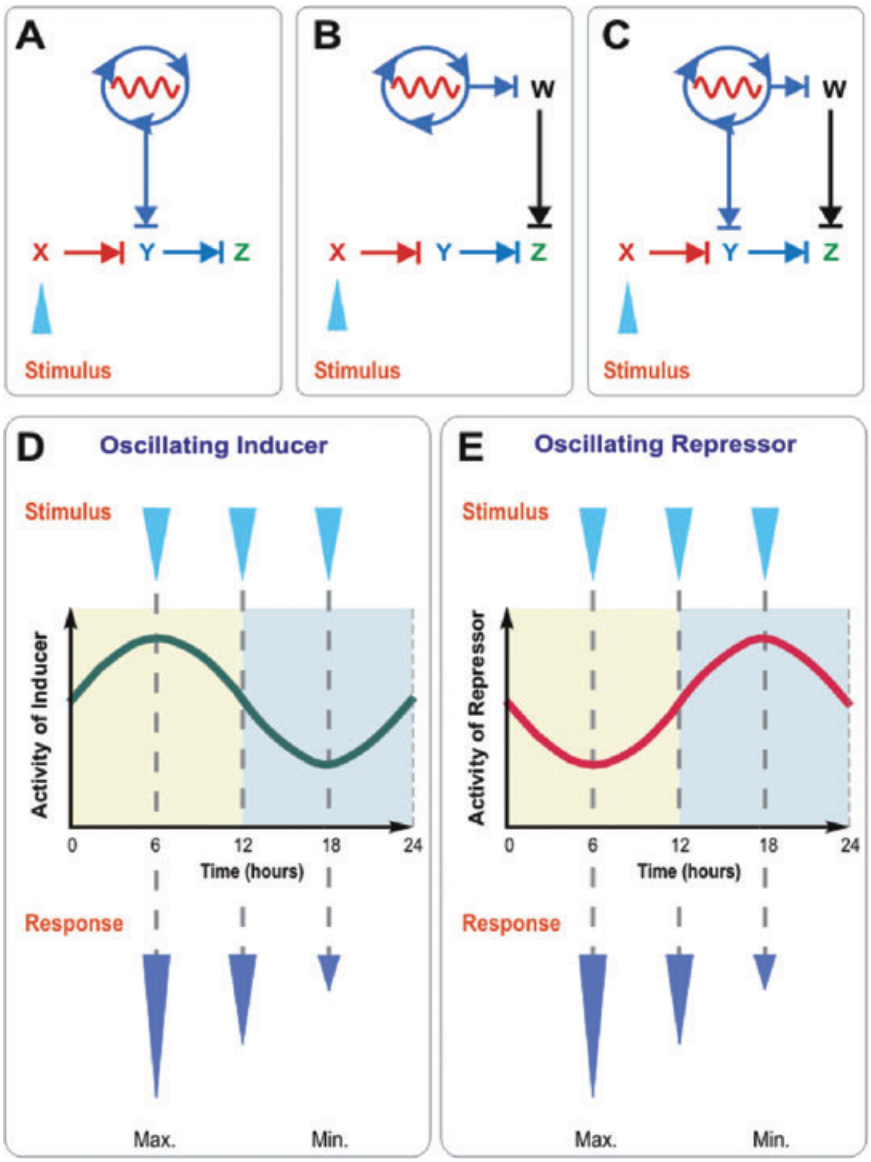

Figure 3. Mechanisms of the gating of plant responses to stimuli by the circadian clock. The circadian clock may gate responses to external stimuli by regulating one or more component(s) of (a) a pathway involved in the transduction of a stimulus, (b) a separate gating pathway that modulates any component of the stimulus-coupled signalling pathway, or (c) a combination of both stimulus-coupled signal transduction pathways and distinct gating pathways. In a–c, all the interactions could be negative or positive and are therefore indicated using arrows with perpendicular lines to indicate both possibilities. Although the circadian clock may regulate any number of components of the pathway(s), rhythmic regulation of any one rate-limiting step may be sufficient to gate the response. In this model, the variation in plant responses to the same stimulus applied at different times of the day is the result of the relative levels of the expression or activity of the regulatory component. This component may be (d) an activator and/or (e) a repressor of a step in the signalling pathway. Thus, a large response is enabled when an activator is at a maximum level and a smaller response when it is at a minimum (d). Furthermore, a repressor that is expressed in an opposite phase of this activator would generate the same pattern of responses (e). Similarly, anti-phased regulation of both repressor and activator would also generate a gated output.

EARLY-FLOWERING 3 (*ELF3*), the intensity of *CAB2* response to light is similar to the maximal response found in wild-type plants, which suggests that in the wild type, ELF3 represses this pathway in a rhythmic manner (Fig. 4d; McWatters *et al.* 2000). In addition, the null mutant *elf3-1* is arrhythmic for many circadian outputs after 10 h in LL, but not in DD, which suggests that gating of light signalling is important in the maintenance of free-running circadian rhythms under LL (Hicks *et al.* 1996; McWatters *et al.* 2000; for a review, see Carré 2002). Many of the *elf3*-1 phenotypes, such as pale leaves and long hypocotyl and petioles, are associated with defective light responses (Covington *et al.* 2001; Hicks, Albertson & Wagner 2001; Liu *et al.* 2001). A two-hybrid screen suggested that ELF3 could act through interaction with the C-terminal domain of PHYB (Liu *et al.* 2001). However, *elf3*-1 and *phyB*-1 mutations are additive in hypocotyl elongation, which suggests that ELF3 may function independently of PHYB (Reed *et al.* 2000). Mutation of *TIC* also causes alterations in the gating of light-induced *CAB2* expression, but this is observed at a different time of the cycle. While ELF3 acts with maximal effect during the early parts of the night, TIC acts in the middle to late part of the night (Hall *et al.* 2003). Recently, *FAR-RED ELONGATED HYPOCOTYL 3* (*FHY3*) was associated with the gating of PHY signalling into the circadian clock (Allen *et al.* 2006).

Shade-avoidance responses, such as increases in stem and petiole elongation in response to shading, are important strategies for maximizing light harvesting by plants that grow in highly populated areas (Schmitt *et al.* 2003). Light quality alters when sunlight passes through, or reflects from the leaves of other plants. As far-red (FR) is poorly absorbed by plants, when other plants are nearby or covering a plant, the FR portion of the spectra is enriched compared with the red (R). The ratio between R and FR (R : FR) in daylight is usually ≈ 1.15 and drops to 0.05–0.7

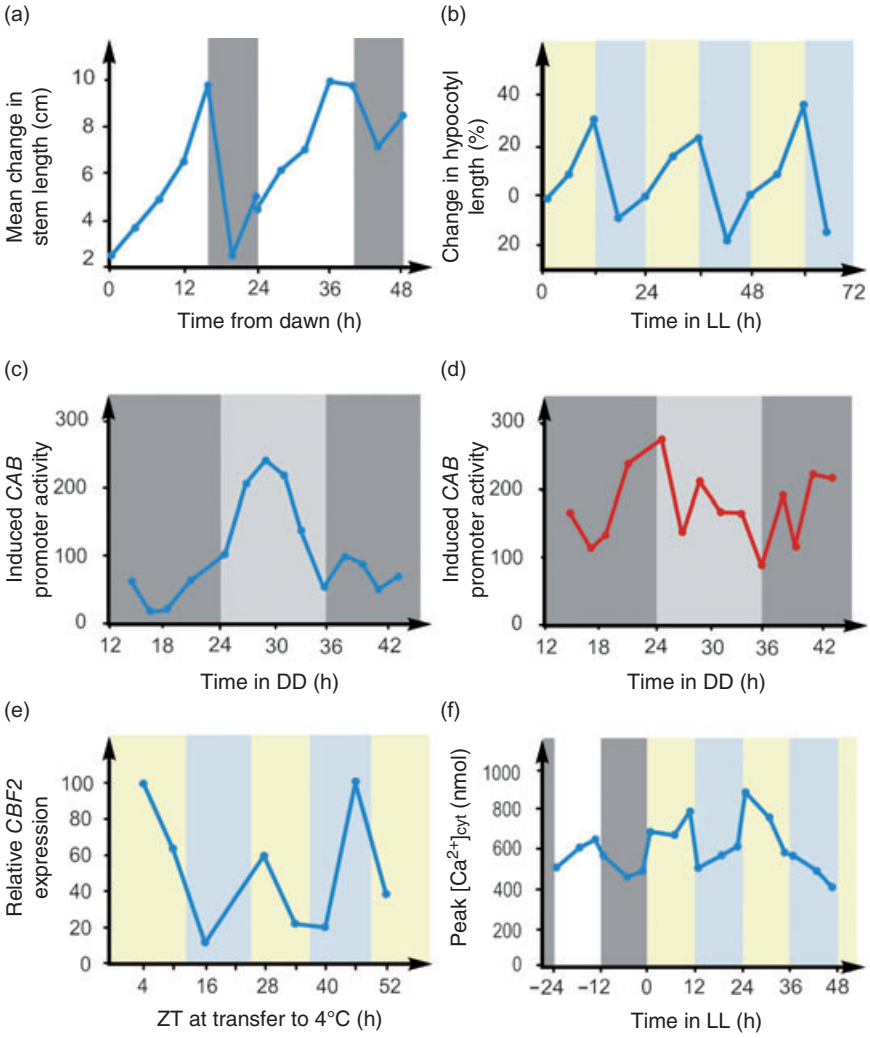

Figure 4. Gating of responses to environmental stimuli. The extent of a plant's response to many environmental stimuli depends on the time of day at which the stimulus is applied. (a) Inhibition of stem growth by mechanical stimulation in *Phaseous vulgaris* in light/dark cycles (LD) is greatest when the stimuli is applied during the night (data redrawn from Anderson-Bernadas *et al*. 1997; © 1997, with permission from Elsevier). (b) Gating of low R : FR-induced hypocotyl elongation. Plants in continuous light (LL) were exposed to low R : FR for 2 h at different points in the subjective day and night. The mean change in hypocotyl length after 24 h is illustrated (adapted from Salter *et al*. 2003 by permission from Macmillan Publishers Ltd., © 2003). (c) Gating of the acute induction of *CHLOROPHYLL A/B BINDING PROTEIN::LUC* (*CAB::LUC*) by light in *Arabidopsis*, and (d) the effect of the *elf3–7* mutation (red) on the gating of the acute induction of *CAB::LUC* luminescence. Entrained plants growing in constant darkness (DD) were exposed to 20 min pulses of white light. The induction of *CAB::LUC* luminescence after subtraction of the resting luminescence signal is shown. The acute induction of *CAB::LUC* luminescence is greatest in the middle of the subjective day but gating is absent in *elf3–7* (adapted from McWatters *et al*. 2000 by permission from Macmillan Publishers Ltd., © 2000). (e) Gating of the low-temperature induction of *CBF2* expression. Plants in LL were exposed to low temperatures (4 °C) at varying times during the subjective day. The levels of *CBF2* expression were determined by RNA blot analysis (redrawn from Fowler *et al*. 2005; © 2005 with permission from American Society for Plant Biologists). (f) Gating of low temperature-induced $[Ca^{2+}]_{cyt}$ increases. Plants in LD or LL were exposed to low temperatures, and the levels of $[Ca^{2+}]_{cyt}$, as measured by aequorin luminescence, were recorded. The extent of induction of $[Ca^{2+}]_{cyt}$ by cold during diurnal and circadian time-courses is shown (redrawn from Dodd *et al*. 2006; © 2006 with permission from Blackwell Publishing). White and dark grey bars in LD represent light and dark periods, respectively. Light yellow and blue bars represent subjective day and night in LL. Light grey and dark grey bars represent subjective day and night in DD. $[Ca^{2+}]_{cyt}$, cytosolic-free calcium; *CBF2*, *C-REPEAT BINDING FACTOR*; *elf3–7, early-flowering 3–7*; R : FR, ratio between red and far-red.

underneath canopies of vegetation (Smith 1982). Consequently, a low R : FR signals shading by other plants. Plants can estimate R : FR of incoming light through the interconversion of PHY between a red-absorbing, biologically inactive form (Pr) and a far-red-absorbing, biologically active form (Pfr). The R : FR ratio of the incoming light is perceived by the plant as a ratio between the Pr and Pfr forms of PHY. Low R : FR will reduce the amount of the active Pfr form of the PHY, which leads to a series of photomorphogenic events, the shade-avoidance responses,

usually at the expense of leaf and storage organ development (for a review, see Franklin & Whitelam 2005). Shade-avoidance responses are also modulated by the circadian clock (Fig. 4b; Salter, Franklin & Whitelam 2003). *PIF3-LIKE 1* (*PIL1*), a component necessary for hypocotyl elongation in shade-avoidance responses, is one of the genes most highly up-regulated in response to a low R : FR (Salter *et al*. 2003). *PIL1* transcript levels oscillate with a circadian period under low, but not under high R : FR, with a peak at dawn. This coincides with the peak of PHYA protein levels (Sharrock & Clack 2002). Hypocotyl growth in response to low R : FR, on the other hand, occurred maximally when the stimulus was given at dusk (Fig. 4b; Salter *et al*. 2003), which coincides with the maximum rate of cell expansion (Dowson-Day & Millar 1999; Harmer *et al*. 2000).

Gating of stomatal responses to the environment

Regulation of the size of the stomatal pore, during favourable environmental conditions, optimizes CO_2 uptake against water loss. During conditions of stress, however, stomatal closure prevents water loss. In order to control stomatal aperture, the stomatal guard cells integrate many internal and external signals and produce an appropriate turgor response that results in guard cell movements (Hetherington & Woodward 2003). The circadian clock is one of the many regulators of stomatal aperture in well-watered plants (see Webb 1998, 2003 for reviews). In C3 plants, the stomatal pore is opened wider during the subjective day than during the subjective night in LD, LL (Martin & Meidner 1971; Hennessey & Field 1991, 1992; Dodd, Parkinson & Webb 2004) and DD cycles (Stålfelt 1963; Martin & Meidner 1972; Heath 1984; Holmes & Klein 1986; Hennessey, Freeden & Field 1993). However, rhythms under DD show considerable damping after the first two cycles (Holmes & Klein 1986). In addition, during LD cycles, stomatal aperture can anticipate both light-to-dark and dark-to-light transitions (Somers *et al*. 1998b; Webb 1998; Dodd *et al*. 2004, 2005b). Each guard cell probably contains its own circadian oscillator because mature guard cells are symplastically isolated and rhythms of guard cell movements persist in detached epidermis (Gorton *et al*. 1989).

Circadian anticipation of dawn in C3 and C4 plants promotes stomatal opening, which allows CO_2 uptake and fixation as soon as sufficient light is available to drive photosynthesis. Stomata stop opening around midday and start closing long before dusk (Webb 1998). These responses were traditionally considered a consequence of the water status of the leaf, but recent work demonstrates that, at least in well-watered plants, they are due to circadian control of the guard cell (Dodd *et al*. 2005b). Under LD cycles, the stomata of arrhythmic *CCA1*-ox plants did not anticipate dawn and continued to open for the entire light period. Furthermore, *CCA1*-ox plants had no pre-dusk closure, which demonstrates that these responses are a consequence of circadian control (Dodd *et al*. 2005b). Consequently, the *CCA1*-ox lines used more water, which suggests that circadian control of stomatal movements provides advantage by increasing water-use efficiency (Dodd *et al*. 2005b).

The intensity of the stomatal response to light depends on the time of the day the stimulus is given, which provides evidence of circadian gating of stomatal responses (Martin & Meidner 1971; Gorton, Williams & Assman 1993; Webb 1998). Stomatal aperture increases in response to white, red and blue light. The stomatal responses to blue light are mediated by PHOT (Kinoshita *et al*. 2001, 2003) and, possibly, the carotenoid zeaxanthin (Frechilla *et al*. 1999; Talbott *et al*. 2003). However, the basis of stomatal responses to red light is less clear. PHY-mediated stomatal opening could be observed in *npq1*-2, a zeaxanthin mutant, but not in the wild type (Talbott *et al*., 2003). The establishment of a link between PHY and stomatal movements has been controversial (Karlsson 1988), and many of the responses to red light have been attributed to a photosynthetic component of stomatal regulation.

Sensitivity to red, blue and white light is maximal in the early to the middle of the subjective day and less effective during the subjective night (Dodge, Marsh & Tallman 1992; Gorton *et al*. 1993). In contrast, dark is more effective at closing the stomata during the subjective night in LL (Martin & Meidner 1971). Green light was not thought to be a biologically active signal in plants, but there is accumulating evidence that green light can reverse the effects of blue light. This may be physiologically significant because foliage cover removes more blue than green light (Klein 1992). Blue light induced stomatal opening, which is maximal in the morning, can be reversed by green light (Talbott *et al*. 2006). Guard cells are most sensitive to green light in the morning and are almost insensitive to green light during the rest of the day (Talbott *et al*. 2006).

Other stimuli such as indole-3-acetic acid (IAA), K^+ anions and fusiccocin are less effective at inducing stomatal opening during the night in the C3 plant *Commelina communis* than during the day (Snaith & Mansfield 1985, 1986). The signalling molecule abscisic acid (ABA) promotes stomatal closure but is less effective when given in the early to middle part of the subjective day (Correia *et al*. 1995). The rhythmic sensitivity to ABA favours CO_2 uptake in the morning before water becomes a limiting factor in the late afternoon. Circadian gating also probably allows stomata to respond appropriately to signals in a phase-specific manner. For example, in C3 and C4 plants, high levels of leaf auxin, in the absence of circadian gating, would promote stomatal opening at night, resulting in water loss with no gain in carbon fixation (Webb 1998).

Gating of responses to low temperature

In cold acclimation, plants acquire tolerance to freezing temperatures after exposure to non-freezing low temperatures (LT). When *Arabidopsis* plants are exposed to LT, the expression of a family of transcription factors called *C-REPEAT BINDING FACTOR* (*CBF1-3*, also known as

DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEIN 1b, *DREB1c* and *DREB1a*, respectively) are induced rapidly (Vogel *et al.* 2005). The *CBF* genes, in turn, induce transcription of more than 100 genes known as the *CBF* regulon, which confers not only enhanced freezing and chilling tolerance but also salt and drought tolerance (Liu *et al.* 1998; Fowler & Thomashow 2002; Maruyama *et al.* 2004; Vogel *et al.* 2005). However, if the *CBF* regulon is constitutively activated, plants have a reduced growth rate, reduced height, delayed flowering and low seed yield (Liu *et al.* 1998; Kasuga *et al.* 1999; Gilmour *et al.* 2000). In order to avoid those deleterious effects, the *CBF* regulon is very tightly regulated. During continuous exposure to LT, for example, the expression of the regulon is reduced (Zarka *et al.* 2003). *CBF1-3* expression at both basal levels and in response to LT is regulated by the circadian clock (Fowler, Cook & Thomashow 2005). When *Arabidopsis* plants grown at 24 °C were exposed to 4 °C under LL, the maximum *CBF1-3* increase in transcription was observed when the stimulus was given in the early morning (ZT4, Fig. 4e). Similar results were observed in the expression pattern of the *RELATED TO ABA-INSENSITIVE 3/VIVIPAROUS 1* (*RAV1*) transcription factor associated with *CBF1-3*. The expression of *ZINC FINGER (C2H2 TYPE) FAMILY PROTEIN 12* (*ZAT12*), a zinc-finger transcription factor that represses the *CBF1-3* regulon, was highly induced at an opposite phase of *CBF1-3* (ZT16). In *CCA1*-ox lines, no variation in the cold-response was present (Fowler *et al.* 2005).

The mechanisms through which the circadian clock gates responses to LT are unknown, but Fowler *et al.* (2005) suggest that the LT sensor is more sensitive at ZT4 or that the CBF gene promoters have regulatory elements to respond to both low temperature and the circadian clock. Alternatively, the plant circadian clock may gate responses to LT through the regulation of the cold signal transduction pathway. Exposure of *Arabidopsis* to LT evokes increases in the concentration of cytosolic-free calcium ($[Ca^{2+}]_{cyt}$) (Knight *et al.* 1991; Plieth 1999) that, in turn, can induce the expression of CBF-related genes, such as *DESICCATION-RESPONSIVE PROTEIN 29A* (*RD29A*; Henriksson & Trewavas 2003). *RD29A* also responds to ABA and cyclic adenosine diphosphate ribose (cADPR; Viswanathan & Zhu 2002; Wu *et al.*, 2003). $[Ca^{2+}]_{cyt}$ responses to LT are gated by the circadian clock in LD and LL, with a maximum response at the middle of the day (Fig. 4f; Dodd *et al.* 2006). Both basal and LT-induced levels of *RD29A* expression are circadian-regulated and they correlate with the gating of LT-induced $[Ca^{2+}]_{cyt}$ increases (Dodd *et al.* 2006).

CIRCADIAN $[Ca^{2+}]_{cyt}$ OSCILLATIONS ENCODE ENVIRONMENTAL INFORMATION

In plants, changes in $[Ca^{2+}]_{cyt}$ are involved in the transduction of many signals, such as ABA, auxin, CO_2, blue and red light, heat, cold, and salt stress, touch and pathogen attack (Hetherington & Brownlee 2004). The stimulus-induced increases in $[Ca^{2+}]_{cyt}$ have great variation in dynamics, including sustained oscillations, some of which can encode information (Evans, McAinsh & Hetherington 2001; Dodd *et al.* 2006). In addition to stimulus-induced oscillations of $[Ca^{2+}]_{cyt}$, which have oscillation periods in the order of minutes, $[Ca^{2+}]_{cyt}$ also oscillates with a period of 24 h in LL, LD and DD, which is indicative of circadian control (oscillations in DD occur in tobacco but not in *Arabidopsis*; Johnson *et al.* 1995; Wood *et al.* 2001; Love, Dodd & Webb 2004). The peak of the 24 h oscillations of $[Ca^{2+}]_{cyt}$ happens during the subjective day, but the exact timing depends on the photoperiod during entrainment. In short days (8 h light/16 h dark, 8L/16D) $[Ca^{2+}]_{cyt}$ peaks 6–8 h after dawn, and the peak coincides with dusk, whereas in long days (16L/8D) the peak is a few hours later but $[Ca^{2+}]_{cyt}$ has returned to 'resting' concentrations by dusk (Love *et al.* 2004). Thus, there is potential for photoperiodic information to be encoded in the phase of circadian and LD oscillations of $[Ca^{2+}]_{cyt}$ (Love *et al.* 2004). The amplitude of circadian oscillations of $[Ca^{2+}]_{cyt}$ increased as the light intensity increased, suggesting that information about photon flux density could also be encoded in circadian oscillations of $[Ca^{2+}]_{cyt}$ (Love *et al.* 2004).

It is striking that there is circadian regulation of $[Ca^{2+}]_{cyt}$ (Johnson *et al.* 1995), circadian gating of cold-induced increases in $[Ca^{2+}]_{cyt}$ (Dodd *et al.* 2006) and that many of the abiotic and biotic stimuli, whose responses are known to be gated by the circadian clock, can signal through alterations in $[Ca^{2+}]_{cyt}$ (e.g. ABA, IAA, blue and red light; Hetherington & Brownlee 2004). This may suggest that $[Ca^{2+}]_{cyt}$ sits at the centre of a network integrating temporal and environmental information. Alternatively, it suggests that gating of stimuli may occur early in the signalling cascades. Until more is known about the mechanisms by which the circadian clock regulates $[Ca^{2+}]_{cyt}$ and participates in gating, we can only speculate as to the architecture of this network (for reviews, see Dodd, Love & Webb 2005a; Gardner *et al.* 2005).

CIRCADIAN CLOCK AND SEASONAL RESPONSES

The circadian clock may act as a reference that allows plants to measure changes in the timing of external events. This is especially useful in latitudes where day length varies through the year. The ability to detect changes in day length permits anticipation of seasonal changes and induction of responses such as cold acclimation and flowering. The detection of changes in day length and the associated responses are called photoperiodism. Many physiological processes, such as bud dormancy, tuber and bulb formation, frost tolerance and flowering, are dependent on photoperiodism (Thomas 1998).

The regulation of flowering time is a good model of how photoperiodic mechanisms might work in *Arabidopsis* (see Hayama & Coupland 2004; Baurle & Dean 2006 for reviews). The perception of day length in *Arabidopsis* depends on the expression of *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) and their relative phase to the LD cycle (Roden *et al.* 2002; Yanovsky & Kay 2002;

Imaizumi *et al*. 2003). *CO* transcription is regulated by two components: GI, which provides a direct input from the circadian oscillator; and FKF1, which integrates both circadian and photoperiodic signals (Imaizumi *et al*. 2005; Mizoguchi *et al*. 2005). CO, in turn, directly induces *FT*, a flowering elicitor (Samach *et al*. 2000). *GI* appears to affect flowering time by regulating the phase and period of the oscillator but may also have clock-independent effects on flowering (Mizoguchi *et al*. 2005). Under short days, transcripts of *FKF1* and *CO* peak in the dark phase. In contrast, under long days, *FKF1* peaks during the light phase. Light activates FKF1, which targets CYCLING DOF FACTOR 1 (CDF1) for degradation. CDF1 represses *CO* expression. Thus, when CDF1 is degraded, *CO* expression levels are increased and *CO* transcript abundance, like *FKF1*, peaks before dusk (Suárez-López *et al*. 2001; Imaizumi *et al*. 2005). Control of *CO* also occurs at the post-transcriptional level as lines that transcribe *CO* constitutively still have rhythms in CO protein levels (Valverde *et al*. 2004). CO protein degradation is mediated by PHYB in the morning. In contrast, PHYA and CRYs counteract PHYB action in the afternoon, stabilizing CO proteins (Valverde *et al*. 2004). Therefore, CO proteins are only accumulated when both *CO* transcripts levels are high and the translated protein is stabilized by PHYA and CRYs. Finally, high levels of CO proteins activate the expression of *FT*, which promotes flowering (Hayama & Coupland 2004). It is not clear how the PHYA and CRYs counteracts PHYB action in flowering-time control, but gating of light signals is likely to be important in this process. In order to stabilize CO levels, PHYA must be present late in the afternoon. However, even though *PHYA* expression peaks at this time, PHYA protein is photolabile and only accumulates during the night (Tóth *et al*. 2001; Sharrock & Clack 2002).

HOW DOES THE CIRCADIAN CLOCK INCREASE FITNESS?

The complexity of interactions between the circadian clock and the environment suggests that a gain of fitness might be conferred by these signalling mechanisms. Circadian clocks have arisen many times during evolutionary history, with a certain degree of convergence, which may indicate that a functional circadian clock with certain characteristics results in selective advantage (Dunlap 1999; Young & Kay 2001). However, there are surprisingly few clear measurements of the fitness benefits conferred by a correctly operating oscillator. When the period of the circadian clock (τ) is similar to the period of the environment (T), it is said that both rhythms are resonant. The selective advantages conferred by the clock, however, are most clearly seen in non-resonant cycles, when τ mismatches T. As an example, tomato plants grew higher, with greener and larger leaves, under 12L/12D cycles ($T = 24$ h) than the plants grown under 6L/6D cycles ($T = 12$ h) or 24L/24D cycles ($T = 48$ h), even though the total amount of light in all treatments was equal (Highkin & Hanson 1954). More striking are the experiments using cyanobacteria or *Arabidopsis*-period mutants under cycles with different T (Ouyang *et al*. 1998; Woelfle *et al*. 2004; Dodd *et al*. 2005b). When strains of cyanobacteria with different τ (22 h, 25 h or 30 h) were grown together under 11L/11D cycles ($T = 22$ h), the short-period mutant strain ($\tau = 22$ h) outgrew the other strains. In contrast, under 15L/15D cycles ($T = 30$ h), the dominating strain was the one with a longer period and, similarly, the dominating strain in 24 h cycles ($T = 24$ h) was the wild-type strain ($\tau = 25$ h; Ouyang *et al*. 1998). Circadian resonance was also found to be important in *Arabidopsis* (Dodd *et al*. 2005b). Both the short-period mutant *toc1*-1 ($\tau \cong 21$ h) and the long-period mutant *ztl*-1 ($\tau \cong 28$ h) had enhanced fitness traits (biomass, photosynthesis and competitive advantage) when grown in environmental rhythms that were matched to their endogenous rhythms. The mutant *toc1*-1 performed best in an environmental rhythm of 10L/10D ($T = 20$ h) and *ztl*-1 did best in 14L/14D ($T = 28$ h). Similarly, the wild type did better in 12L/12D ($T = 24$ h) than in 20 h or 28 h cycles. Furthermore, arrhythmic *CCA1*-ox performed worse than the wild types grown under 12L/12D (Dodd *et al*. 2005b). *CCA1*-ox lines also produce less viable seed under very short days (Green *et al*. 2002), but the interaction of the circadian clock with the flowering-time apparatus makes the interpretation of such a result difficult.

The circadian clock is likely to increase the fitness of plants through many mechanisms, including (1) temporal compartmentation of metabolic processes; (2) anticipation of daily environmental changes; (3) optimization of the turnover rate of proteins; (4) anticipation of seasonal environmental changes; and (5) gating of environmental signals. We discuss each of these possibilities in turn.

1 *Temporal compartmentation of metabolic processes.* The circadian clock allows organisms to separate the occurrence of two incompatible mechanisms in time. Temporal organization can protect biochemical pathways that are photoinhibited or easily photodamaged by light (Pittendrigh 1993). The most striking example of temporal compartmentation found in plants is Crassulacean acid metabolism (CAM). CAM plants fix CO_2 through two mechanisms that are separated in time but not space (for reviews, see Borland & Taybi 2004; Hartwell 2005). In CAM plants, the stomata open during the night when PHOSPHOENOLPYRUVATE CARBOXYLASE (PEPC) fixes CO_2, producing malic acid, which is stored inside the vacuole. During the day, the malic acid is released from the vacuole and is decarboxylated. The released CO_2 accumulates inside the mesophyll, causing stomatal closure, while ribulose bisphosphate carboxylase oxygenase (Rubisco) refixes the CO_2, as in C3 plants (Hartwell 2005). As the stomata are closed during the day and opened during the night, water loss is reduced. In order to prevent futile cycles of carbon, the timing of the metabolic pathways involved in CAM biochemistry is regulated. Several orthologues of the *Arabidopsis* circadian clock genes are found in the CAM plant *Mesembryanthemum crystallinum*, but the mechanisms by which the temporal control of CAM is brought about are still under

investigation (Boxall *et al*. 2005). CAM photosynthesis is energetically expensive, but it results in increased water-use efficiency. Moreover, the accumulation of CO_2 because of malic acid decarboxylation inside the mesophyll inhibits photorespiration. In the balance between the energetic costs of CAM and the benefits provided by it, there is an increase of selective advantage in environments where water availability and photorespiration are key selective pressures (Borland & Taybi 2004).

2 *Anticipation of daily environmental changes.* Rhythms of leaf movements, driven either by differential growth changes in the upper and lower part of the *Arabidopsis* petiole or by turgor changes in the pulvini of legumes (for a review, see Webb 2003), may increase photosynthetic rates by enabling tracking of the sun during the morning (Pastenes, Pimentel & Lillo 2005). More importantly, when sunlight is at its greatest intensity, in the middle of the day, legume leaves stay almost vertical, thus reducing sunlight absorption and avoiding photodamage, overheating and increased water loss (Pastenes *et al*. 2005). Water loss is also prevented by the anticipation of dawn and dusk by the stomatal guard cells (see 'Gating of stomatal responses to the environment'; Dodd *et al*. 2005b). The synthesis of phenylpropanoids before dawn allows the preparation of ameliorative mechanisms before the onset of photodamage (Harmer *et al*. 2000). The anticipation of dawn is another key process for the plant as the photosynthetic apparatus can assemble prior to light availability. A correctly functioning clock allows plants to accumulate more chlorophyll and fix more carbon, which results in 45% more biomass, faster growth and lower mortality (Dodd *et al*. 2005b).

3 *Optimization of the turnover rate of proteins.* The timing of protein synthesis might also optimize protein turnover when there is a periodic increase of protein damage. During light-harvesting, many proteins and pigments are photodamaged. The time of highest expression of proteins related to the light-harvesting complex and other related proteins coincides with the timing of higher photon flux in the day (Millar & Kay 1996; Harmer *et al*. 2000). The timing of expression of those proteins and pigments results in almost uniform amounts of those components during the light phase (Prombona & Argyroudi-Akoyunoglou 2004). Indeed, plants are chlorotic under photoperiods with light phases longer than 18 h (Withrow & Withrow 1949) and have lower chlorophyll content in non-resonant conditions (Dodd *et al*. 2005b).

4 *Anticipation of seasonal environmental changes.* Besides the advantages of freezing tolerance development, bulbs and tuber formation, bud dormancy and other phenomena related to winter avoidance and tolerance, photoperiodism also allows the synchronous flowering of plant species, which enhances outbreeding and therefore increases genetic recombination. At the beginning of spring, photoperiodism can also allow small plants to exploit seasonal niches. In the temperate woodlands, some fast-growing plants can use the days before the leaf canopy is formed to grow, flower and set seed (Thomas 1998). In addition, different *Arabidopsis* ecotypes have a wide range of free-running circadian periods, which are positively correlated with the latitude of their collection (Michael *et al*. 2003b). It is perhaps counter-intuitive that there may be selection pressure for clock periods greater than 24 h at high latitudes, because circadian periods greater than the environmental rhythm can be disadvantageous to plants (Highkin & Hanson 1954; Dodd *et al*. 2005b). However, circadian clocks with periods not exactly matched to the period of the environment are associated with more flexible and accurate entrainment mechanisms on long days (Roennenberg, Daan & Merrow 2002). Longer circadian periods, which deviate from environmental rhythm periods, may be expected in latitudes where day length varies greatly to optimize photoperiodic perception. Furthermore, a longer rhythm is required for the correct phasing of metabolic pathways under days with very long light phases (Michael *et al*. 2003b).

5 *Gating of environmental signals.* Gating might contribute to the optimization of environmental signal processing. Previously, it has been suggested that gating of stomatal responses to ABA and auxin increases water-use efficiency by allowing responses that favour CO_2 uptake in the day while minimizing water loss at night (Webb 1998). The gating mutant *elf3-1* is less viable than the wild type, in both long days (16L/8D) and very short days (4L/20D) and performs even worse than the circadian arrhythmic lines *CCA1*-ox and *LHY*-ox in the same conditions (Green *et al*. 2002). These data are difficult to interpret because early flowering time, and other pleiotropic effects, may affect seed quality independently of the circadian clock, but they do provide evidence that the gating of responses to the environment is a fundamental component in plant and environment interactions and is essential for the success of land plants.

The circadian clock structure and function reflect the characteristics of the rhythmic environment in which they evolved. Entrainment mechanisms that continuously adjust the phase of the circadian clock in relation to the environment were selected because the day length is continuously changing. Photoperiodism was selected in response to seasonal changes that could be predicted by measuring changes in day length. The circadian clock is the result of the selective pressure from a rhythmic environment that continuously challenges organisms with predictable environmental changes.

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