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Interactions between circadian and hormonal signalling in plants

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Abstract Growth and development of plants is controlled by external and internal signals. Key internal signals are those generated by hormones and the circadian clock. We highlight interactions between the circadian clock and hormonal signalling networks in regulating the physiology and growth of plants. Microarray analysis has shown that a significant proportion of transcripts involved in hormonal metabolism, catabolism, perception and signalling are also regulated by the circadian clock. In particular, there are interactions between the clock and abscisic acid, auxin, cytokinin and ethylene signalling. We discuss the role of circadian modulation ('gating') of hormonal signals in preventing temporally inappropriate responses. A consideration of the daily changes in physiology provides evidence that circadian gating of hormonal signalling couples the rhythmic regulation of carbon and water utilisation to rhythmic patterns of growth.

Keywords Circadian clock · Hormone · Abscisic acid · Auxin · Cytokinin · Ethylene · Calcium · Cold · Arabidopsis

Introduction

The circadian clock is an internal timekeeper that is an adaptation to day/night cycles providing, in *Arabidopsis thaliana*, competitive advantage (Dodd et al. 2005) and probably an increase in fitness (Green et al. 2002). Circadian clocks regulate many processes in plants, including leaf and stomatal movements, photosynthesis and growth and they also contribute to photoperiodism (Gardner et al. 2006; Hotta et al. 2007; Imaizumi and Kay 2006). In recent years the importance of circadian regulation of signalling pathways in the daily life of plants has been demonstrated by studies showing that the circadian clock modulates (or 'gates') signalling events (Covington and Harmer 2007; Dodd et al. 2006; Fowler et al. 2005; Hotta et al. 2007; Salter et al. 2003). Gating of a response by the clock results in variations in the intensity of that response depending on the time of day that the initial stimulus was applied. The molecular mechanisms by which gating occurs are unknown but one possibility is that one or more components involved in the transduction of the signal are circadian-regulated (specific examples of gating and a discussion of the possible mechanisms by which it could occur are in Figs. 2, 3 and 4 of Hotta et al. 2007).

Circadian gating demonstrates that there is an interaction between the circadian and hormonal signalling networks. System-wide analyses have suggested that the interactions between hormonal and circadian signalling might be considerable. In *A. thaliana*, estimates of the circadian control of gene expression from microarray analyses of oscillating transcript abundance in constant light vary from about 6% to 15% of the genome (Dodd et al. 2007; Edwards et al. 2006; Harmer et al. 2000). Within these datasets there is considerable overlap between circadian-, auxin- and abscisic acid (ABA)-regulation of transcripts (Dodd et al. 2007; Covington and Harmer 2007). The number of rhythmically regulated transcripts is probably even higher than the above estimates. Analysis of the regulation of transcripts, in various combinations of constant and rhythmic conditions and entrainment, demonstrated that 89% of detected transcripts can be rhythmic, when all conditions are considered

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(Michael et al. 2008). These studies suggest that rhythmic regulation of transcripts, including those transcripts involved in hormonal metabolism, catabolism, perception and signalling, is the norm, indicating a strong influence of time-of-day on hormonal responses.

The *Arabidopsis thaliana* circadian clock

An autonomous circadian timekeeper is probably present in every cell of *A. thaliana*. The structure of the *A. thaliana* circadian clock is complex and the distinctions between input pathways that entrain the clock, the central oscillator that generates rhythms and the output pathways controlling physiology, are blurred. The *A. thaliana* circadian clock consists of a series of interconnecting feedback loops that, in addition to regulating outputs, also gate both inputs to the oscillator and other signalling pathways (Fig. 1; Gardner et al. 2006; Hotta et al. 2007). The high degree of interconnectivity between components provides robustness to the molecular oscillator (Locke et al. 2006; Zeilinger et al. 2006).

The phase of the circadian clock is adjusted to match the day/night cycle by entrainment through the phytochromes and cryptochromes (Millar 2004; Salomé and McClung 2005). Light input to the circadian oscillator is important in the morning to match the phase of the internal oscillator

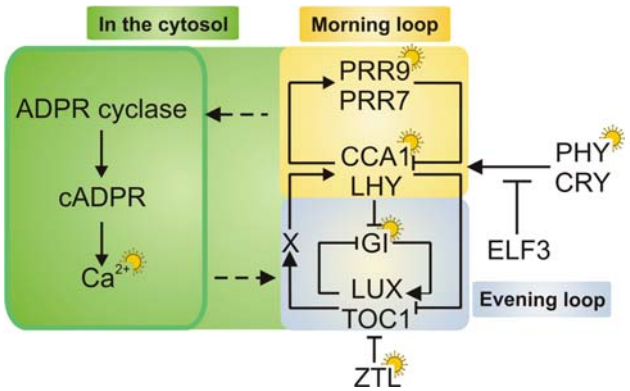

Fig. 1 The circadian clock of *Arabidopsis thaliana*. The central oscillator consists of multiple interlocking transcriptional feedback loops and the small signalling molecules cADPR and Ca^{2+} act in the cytosol to form another feedback loop. Components of the clock associated with light perception are labelled with a sun. Phytochromes (PHY) and cryptochromes (CRY) are the principal photoreceptors that mediate light input to the clock and can themselves be regulated by the clock. *ELF3* is also circadian regulated and gates light input to the clock (Covington et al. 2001; McWatters et al. 2000). Component X is predicted from mathematical modelling (Locke et al. 2005). *Arrows* indicate positive relationships and *bars* negative relationships. The mechanism by which the clock controls oscillations of cADPR and the means by which cADPR and Ca^{2+} input back into the clock are unknown; these relationships are therefore indicated by dotted lines. LUX is LUX ARRHYTHMO (Hazen et al. 2005), all other abbreviations are described in the text

with dawn and in the morning and evening to allow for day length measurement. At dawn, light signals activate a loop of morning-expressed genes consisting of *LHY* (*LATE ELONGATED HYPOCOTYL*), *CCA1* (*CIRCADIAN CLOCK ASSOCIATED1*), *PRR9* (*PSEUDORESPONSE REGULATOR9*) and *PRR7* (Locke et al. 2006; Zeilinger et al. 2006). LHY and CCA1 are two light-activated MYB-like transcription factors that promote the expression of *PRR7* and *PRR9*, two genes of unknown biochemical function. *PRR7* and *PRR9* in turn feedback to repress *LHY* and *CCA1*, thus forming a negative feedback loop active in the morning.

LHY and CCA1 proteins accumulate through the day and repress another loop of the clock composed of evening-expressed genes. In the afternoon, LHY and CCA1 proteins are degraded relieving the repression of expression of *GI* (*GIGANTEA*) and *TOC1* (*TIMING OF CAB EXPRESSION1/PRR1*) which form a feedback loop in which GI activates *TOC1* expression and TOC1 in turn represses *GI* expression. GI interacts with, and stabilizes ZTL (ZEITLUPE), a component of the E3 ubiquitin ligase that targets TOC1 for degradation (Han et al. 2004; Kim et al. 2007; Más et al. 2003). ZTL is unusual in this pathway because *ZTL* transcripts are not under circadian control. However, the circadian alterations in GI abundance confer rhythmic changes to ZTL protein levels. The interaction between ZTL and GI is enhanced by blue light, with ZTL acting as the photoreceptor. This leads to increased ZTL stability at the end of the day when GI and TOC1 begin to accumulate (Kim et al. 2007; Más et al. 2003). At night, the GI-ZTL interaction is reduced and ZTL is freed, presumably to target TOC1 for degradation at the end of the night. This, coupled with the activation of *TOC1* expression by GI, results in peak expression of TOC1 in the first half of the night. The loop of evening expressed genes is coupled back to the 'morning' loop by indirect activation of *LHY* and *CCA1* expression by TOC1 (Locke et al. 2006; Zeilinger et al. 2006).

The interactions between ZTL and GI, and ZTL and TOC1 occur in the cytosol and are examples of post-translational effects that are critical to the robustness and stability of the clock. Post-translational processes contributing to plant circadian timing include phosphorylation (Daniel et al. 2004), ribosylation (Panda et al. 2002) and proteosome-dependent degradation (Más et al. 2003, Han et al. 2004). Rhythms are therefore likely to be an emergent property of the entire cellular system, incorporating both cell physiology and transcriptional feedback loops. The importance of post-translational cytosolic events in the circadian clock has been emphasized by the presence in *A. thaliana* of a loop that incorporates the cytosolic signalling molecule cyclic adenosine diphosphate ribose (cADPR) (Fig. 1; Dodd et al. 2007). In mammals, cADPR is an

important intracellular Ca^{2+}-releasing agent that acts via the Ca^{2+}-induced Ca^{2+} release ryanodine receptor pathway rather than the inositol-(1,4,5)-trisphosphate ($Ins(1,4,5)P_3$) pathway (Lee 2002). cADPR is metabolised from NAD by the enzyme ADP-ribosyl cyclase and a by-product of this reaction is nicotinamide which can be used to inhibit cADPR production (Sethi et al. 1996). Plants respond to cADPR by releasing Ca^{2+} from the vacuole (Allen et al. 1995) and the ER (Navazio et al. 2001), however the receptors involved and the enzyme producing cADPR have not been characterized at the molecular level. In addition to its role in the circadian clock, cADPR has been implicated in a number of stress related responses (Hunt et al. 2004). Circadian oscillations of [cADPR] are driven by the transcriptional feedback loops of the circadian oscillator. Inhibition of cADPR production by nicotinamide abolishes circadian oscillations of $[Ca^{2+}]_{cyt}$ and lengthens the circadian period of *CCA1, LHY, TOC1* and *CAB2* expression and the circadian period of leaf movement. This indicates that cADPR is required to drive circadian oscillations of $[Ca^{2+}]_{cyt}$ and also modulates the nuclear transcriptional feedback loops of the *A. thaliana* circadian oscillator (Dodd et al. 2007). Recently, it has been demonstrated that nicotinamide lengthens the period of the mammalian circadian clock in a manner similar to that in plants. The mechanism by which this occurs has not been explored but the period-lengthening is independent of the inhibitory effects of nicotinamide on SIRT1, a deacetylase that regulates mammalian circadian clock gene expression by counteracting the acetyltransferase activity of CLOCK (Asher et al. 2008). The similarity of the period lengthening effects of nicotinamide on the plant and mammalian oscillator are striking, because in both plants, and the mammalian suprachiasmatic nucleus, circadian oscillations of $[Ca^{2+}]_{cyt}$ are driven by cADPR-sensitive mechanisms (Ikeda et al. 2003; Dodd et al. 2007). The existence of a loop of cytosolic signalling molecules within the circadian clock is likely to enhance stability and allow for both modulation of the circadian clock by stress signals and also circadian modulation of stress signalling, because both cADPR and $[Ca^{2+}]_{cyt}$ participate in the transduction of biotic and abiotic stresses (Dodd et al. 2007).

It has also been proposed that nyctohemeral (daily) oscillations of $[Ca^{2+}]_{cyt}$ are driven by CAS, a Ca^{2+}-receptor, with $Ins(1,4,5)P_3$ acting as the Ca^{2+}-mobilizing second messenger (Tang et al. 2007). It was proposed that CAS, initially thought to be located in the plasma membrane, senses rhythmic changes in apoplastic $[Ca^{2+}]$ caused by changes in water flux as a consequence of stomatal rhythms (Tang et al. 2007). A central tenet of this hypothesis is that rhythms of $[Ca^{2+}]_{cyt}$ and stomatal movements are in phase. However, in the short circadian period mutant *toc1*-1, $[Ca^{2+}]_{cyt}$ and stomatal rhythms become uncoupled (Xu et al. 2007). In *toc1*-1, stomatal rhythms have a period of ~21 h in constant light (LL, Somers et al. 1998), whilst $[Ca^{2+}]_{cyt}$ rhythms have a period of ~24 h (Xu et al. 2007) suggesting that these two rhythms are not functionally linked. Circadian rhythms of $[Ca^{2+}]_{cyt}$ are insensitive to the phospholipase C inhibitor U73122, but are completely abolished by nicotinamide. This suggests that $Ins(1,4,5)P_3$ does not contribute to oscillations of $[Ca^{2+}]_{cyt}$ in constant conditions (Dodd et al. 2007). A recent report provides evidence that CAS is not located on the plasma membrane but rather is mainly localized to chloroplasts as an integral thylakoid membrane protein with the N-terminus Ca^{2+}-binding region probably on the stromal side (Nomura et al. 2008). Whilst it seems unlikely that CAS acts as a plasma membrane receptor linked to phospholipase C to drive circadian oscillations of $[Ca^{2+}]_{cyt}$, it is probable that CAS has an important function in the generation of rhythmic Ca^{2+} signals because the chloroplast is a source of organellar Ca^{2+} rhythms (Johnson et al. 1995; Sai and Johnson 2002).

The uncoupling of $[Ca^{2+}]_{cyt}$ and stomatal rhythms in the *toc1*-1 mutant as well as the absence of $[Ca^{2+}]_{cyt}$ rhythms in the short circadian period *cca1*-1 mutant, indicates that there are multiple, genetically separable, circadian oscillators in *A. thaliana* (Xu et al. 2007). The different circadian oscillators could be located in the same or different cell types (Gardner et al. 2006; Xu et al. 2007). Cell-type specific expression of the clock component *PRR3* may contribute to the differential behavior of circadian clocks in *A. thaliana* (Para et al. 2007).

Interactions between hormone signalling networks and the circadian clock

The physiological processes controlled by plant hormones overlap considerably with those regulated by the circadian clock, indicating possible cross-talk between circadian and hormonal signalling networks. It was noticed as early as 1937 that the sensitivity of plants to auxin varied over the day (Went and Thimann 1937), indicative of what is now known as circadian gating of auxin signalling (Covington and Harmer 2007). Hormonal-clock interactions are not restricted to auxin but might also include ABA and possibly cytokinins and ethylene.

Gating of responses to ABA

ABA regulates events from seed germination right through to seed formation, affecting both vegetative and reproductive processes (Finkelstein and Rock 2002). ABA is also involved in responses to numerous stresses, including water-deficit, salt, hypoxia and cold and affects responses

to wounding and pathogen attack. The circadian clock influences many of these processes. There is highly significant overlap between the transcripts regulated by the circadian clock or daily light/dark cycles and ABA (Mizuno and Yamashino 2008). Transcripts regulated by the closely related methyl jasmonate signalling pathway also overlap with those regulated in day/night cycles (Mizuno and Yamashino 2008). In contrast, transcripts regulated by ethylene, cytokinin, giberellic acid and brassinosteroids do not, or only weakly significantly, overlap with rhythmically-regulated transcripts in constant light and day/night cycles (Dodd et al. 2007; Mizuno and Yamashino 2008).

Stomatal movements are an example of a physiological process regulated by both the circadian clock and ABA. Rhythmic stomatal movements anticipate dawn and dusk (Fig. 2), and the opening of stomata before dawn is effected by activation of inward K^+ channels (Lebaudy et al. 2008). During water-deficit stress ABA reduces the size of the stomatal pore. When water-deficit stress is mild, ABA is less effective at closing stomata in the morning than in the afternoon (Correia et al. 1995; Fig. 2). This may be an example of circadian gating of ABA signalling which ensures stomata open to facilitate CO_2 uptake in the cool of the morning, when transpiration will be lower, but are closed in the heat of the afternoon if water supply is limiting (Webb 1998).

The ABA signalling pathway includes many components in common with cold signalling and plant responses to cold can include an increase in ABA. As with ABA-signalling, there is evidence that cold-signalling is gated by the circadian clock. One function of the cold-signalling pathway is the induction of freezing tolerance in response to prior exposure to non-damaging low temperatures (LT). In *A. thaliana,* part of the freezing-tolerance mechanism is the LT-induction of the *CBF1-3* transcription factors (*C-REPEAT BINDING FACTOR*s also known as *DEHYDRATION-*

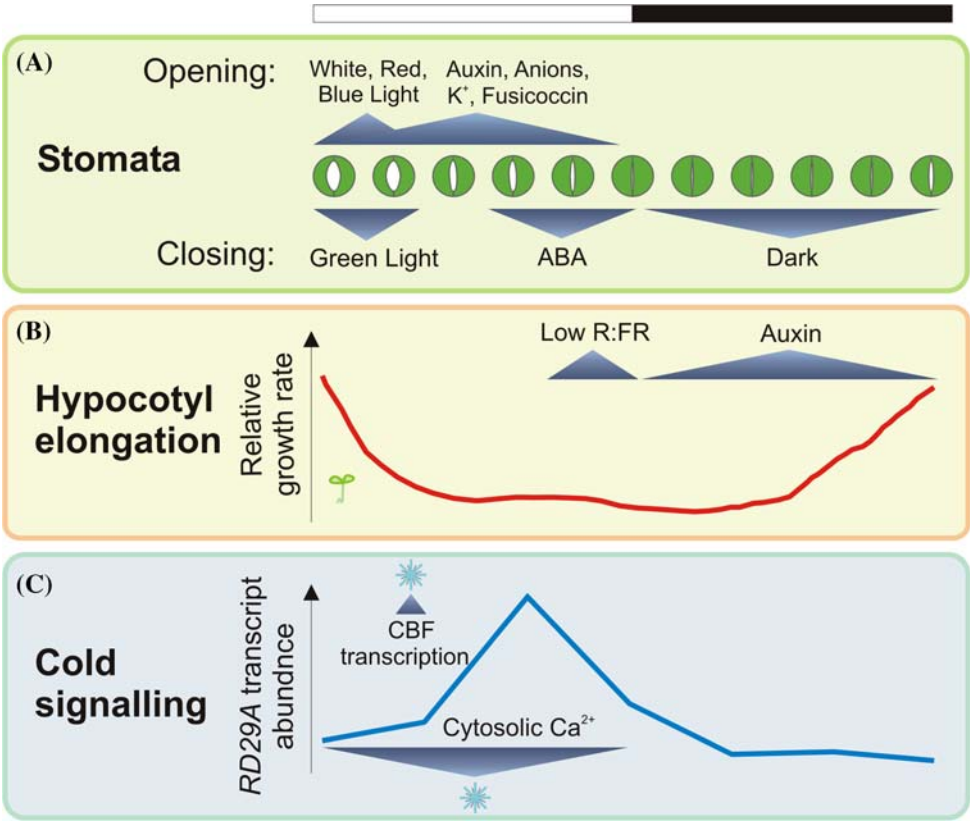

Fig. 2 Gating of responses in stomata, hypocotyl elongation and cold signalling. Stomatal aperture (**a**), relative growth rate of hypocotyl elongation (**b**, modified from Nozue et al. 2007) and *RD29A* transcript abundance (**c**, Smith et al. 2004) are rhythmic in 12:12 LD cycles. The responses of stomata (**a**) and the hypocotyl (**b**) to various stimuli are gated by the clock, with blue triangles showing the approximate times when stimuli have greater effect. **a** Stimuli affecting stomata are divided into those that promote opening and those that promote closure. **b** A Low R:FR ratio and auxin increase hypocotyl elongation maximally at the times indicated. **c** In cold signalling, a LT stimulus (snowflake) can induce transcription of the CBF1-3 transcription factors as well as increases in $[Ca^{2+}]_{cyt}$. These responses are also gated, and the approximate time of the greatest response to LT, in constant light conditions, is indicated by the blue arrows. The experiments summarised in this figure were performed in various photoperiods of light and dark, or constant light and therefore the gating effects might not be directly applicable to a 12:12 LD cycle. To provide a conceptual summary we have included the data from all conditions. A 12:12 LD cycle is indicated by white and black rectangles for reference

RESPONSIVE ELEMENT-BINDING PROTEIN 1b, *DREB1c* and *DREB1a*, respectively; Vogel et al. 2005) that in turn drive the expression of about 200 genes which form the CBF regulon. The *CBFs* also confer tolerance to drought and salt stress (Fowler and Thomashow 2002; Liu et al. 1998; Maruyama et al. 2004). Expression of *CBF1-3* is circadian regulated and induction of *CBF1-3* in response to LT has a 24 h rhythm indicative of circadian gating of LT signalling (Fowler et al. 2005). The maximum LT-induced increase in transcription of *CBF1-3* occurs when plants are exposed to LT 4 h after dawn (Fig. 2) and there is no temporal difference in the LT stimulation of *CBF1-3* expression in arrhythmic *CCA1* overexpressors (*CCA1*-ox). A functional circadian clock is, therefore, required for the rhythmic sensitivity of *CBF1-3* transcription to LT (Fowler et al. 2005).

One arm of the cold-signalling pathway involves ABA-mediated increases in cADPR and $[Ca^{2+}]_{cyt}$ leading to the expression of a suite of cADPR- and Ca^{2+}-responsive genes such as *RD29A* (*RESPONSIVE TO DESICCATION29A*) (also known as *LOW TEMPERATURE INDUCED78* and *COLD REGULATED78*; Viswanathan and Zhu 2002; Wu et al. 2003). The transcript levels of *RD29A*, $[Ca^{2+}]_{cyt}$ and [cADPR] are all circadian-regulated (Dodd et al. 2006, 2007; Johnson et al. 1995). In addition, the acute responses of both *RD29A* and $[Ca^{2+}]_{cyt}$ to cold are gated by the circadian clock, with maximum responsiveness during the subjective day (Dodd et al. 2006; Fig. 2). The involvement of cADPR in a loop of the circadian oscillator is suggestive of a mechanism by which gating of ABA and cold signalling might occur as a consequence of the circadian control of the abundance of the transducing second messengers. Maximal induction by LT of *CBF1-3*, *RD29A* and $[Ca^{2+}]_{cyt}$ during the day might indicate that a different set of responses to cold are required at this time than at night. Because it is usual for the temperature to drop at night and increase during the day, a cold stimulation during the day could be indicative of the onset of autumn and winter. Possibly, long term responses in preparation for winter are required if cold is perceived during the day.

The high degree of interconnection between ABA signalling and the circadian clock is further exemplified by evidence that ABA can affect circadian oscillator period. The period of the rhythmic changes in promoter activity of *CCA1* (a core oscillator gene), *AtGRP7/CCR2* (*GLYCINE-RICH RNA-BINDING PROTEIN7/COLD, CIRCADIAN RHYTHM AND RNA BINDING 2*; a possible slave oscillator component) and *CAB2* (*CHLOROPHYLL A/B-BINDING PROTEIN2*; a marker gene for clock output), are lengthened in response to exogenous application of ABA in LL (Hanano et al. 2006). Consistent with this, the period of *AtGRP7/CCR2* promoter activity rhythms are shortened in the ABA-deficient mutant *aba2* (*abscisic acid absent2*) in constant darkness (Hanano et al. 2006). Whether the effects of ABA on circadian period can be explained due to the effects of ABA on [cADPR] remains to be determined. Transient alterations of [cADPR] have little effect on the oscillator, and prolonged increases in [cADPR] might shorten period (Dodd et al. 2007) in contrast to the reported ABA-induced lengthening of period (Hanano et al. 2006).

Gating of responses to auxin

Auxin is a potent regulator of plant growth and development (Leyser 2005). The levels of both free indole acetic acid (IAA) and conjugated-IAA fluctuate in a circadian manner in *A. thaliana* (Jouve et al. 1999). IAA is required for the circadian rhythms of growth of the flowering stem (Jouve et al. 1999), showing that auxin responses are intimately connected to processes also controlled by the clock. This is also suggested by overlap between some auxin-regulated and circadian transcriptomes (Covington and Harmer 2007; Dodd et al. 2007). Genes involved in every aspect of auxin signalling are circadian-regulated, including biosynthesis, efflux, inactivation and perception (Covington and Harmer 2007). In addition, many auxin-induced and repressed genes are also circadian-regulated (Covington and Harmer 2007). The circadian clock can drive rhythms of expression from a synthetic auxin-responsive promoter, and the clock can gate transcriptional and hypocotyl growth responses to auxin, but auxin has only weak effects on circadian oscillator function (Covington and Harmer 2007). In general, transcriptional and growth responses to auxin tend to peak in the subjective night and be lower during subjective day and this is broadly consistent with the rhythmic growth of the hypocotyl (Fig. 2). In LL, the peak rate of hypocotyl elongation occurs at subjective dusk and growth stops entirely around subjective dawn (Dowson-Day and Millar 1999), whereas in light/dark cycles (LD), the peak of growth is shifted to around dawn (Fig. 2; Nozue et al. 2007). It is not only auxin-induced growth and transcriptional changes that are subject to gating by the circadian clock; stomatal opening can be induced by auxin and the hormone is less effective at inducing stomatal opening in *Commelina communis* during the night than during the day (Fig. 2; Snaith and Mansfield 1985). This is in contrast to the phase in which auxin is most effective in promoting hypocotyl elongation and transcriptional activation, suggesting that different gating mechanisms modulate different responses, possibly through cell-specific components of the circadian gating mechanisms.

Circadian rhythms of ethylene production

The levels of ethylene emission from *A. thaliana* plants oscillate with a circadian period partially due to the

circadian control of the abundance of transcripts encoding 1-amino-cyclopropane-1-carboxylic acid (ACC) synthases (ACSs) which make ACC, the precursor of ethylene (Thain et al. 2004). Among other things, ethylene affects cell growth and shape and is involved in hypocotyl elongation (Abeles et al. 1992) but the function of rhythmic ethylene emission is unclear. Ethylene has no effect on the clock or rhythmic outputs (Thain et al. 2004) and maximal ethylene production in the middle of the day does not coincide with maximal hypocotyl elongation (Nozue et al. 2007). Similarly, ACC has no consistent effects on the oscillator (Hanano et al. 2006). Rhythmic hypocotyl elongation persists in ethylene biosynthesis and signalling mutants, including *etr1*-1, *ein4*-1 and *eto2*-1, suggesting ethylene is not required for rhythmic growth (Thain et al. 2004). However, ethylene has other roles, including involvement in responses to biotic and abiotic stresses, which could represent targets for circadian ethylene signals (Abeles et al. 1992) and these remain to be explored.

Cytokinins can regulate clock function

Cytokinins promote cell division and shoot formation as well as many other growth processes (Sakakibara 2006) and there is some evidence for interactions between cytokinins and the clock (Hanano et al. 2006; Salomé et al. 2006; Zheng et al. 2006). Endogenous application of cytokinin affects the phase but not the period of the clock (Hanano et al. 2006; Salomé et al. 2006; Zheng et al. 2006) and increases transcription of *LHY* and *CCA1* but suppresses transcription of *TOC1* (Zheng et al. 2006). The effect of cytokinin on the clock is thought to be mediated by *ARR4 (ARABIDOPSIS RESPONSE REGULATOR4)*, a gene responsive to cytokinin that also acts as an input to the core oscillator (Salomé et al. 2006). In terms of cytokinin-modulation of circadian rhythms, the *arr4* mutant is insensitive to cytokinin (Zheng et al. 2006) whereas the *ARR4-ox* mutant is hypersensitive to cytokinin (Hanano et al. 2006). Cytokinin and light signalling co-regulate many processes (Thomas et al. 1997) and, as light signalling is gated by the clock (Hotta et al. 2006), it might be informative to further investigate the interactions between cytokinin and circadian signalling.

A day in the life of a plant: physiological significance of interactions between the clock and hormones

Plants gamble the daily consumption of water and carbon against anticipated later replenishment. During the day, C3 and C4 plants loose water when the stomata are open leading to a decrease in water potential. At night, when the stomata are closed, water uptake is greater than water loss

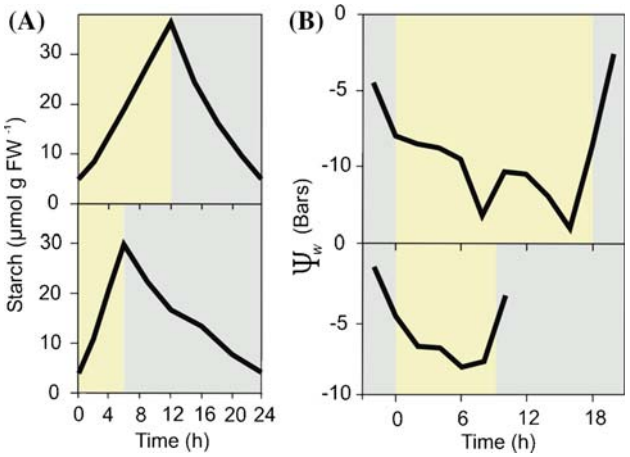

Fig. 3 Daily changes in starch content and water potential. During the day excess photosynthetic carbon is stored as starch in anticipation of night time demand, and at night the starch is metabolised at a constant rate until stores are depleted at dawn (**a**). This basic pattern is maintained, with a period of acclimation, in long and short days (redrawn from Gibon et al. 2004). Water potential (ψ_w) decreases during the day, when stomata are open, and starts to increase around dusk when stomata are more closed in both long and short days (**b**; redrawn from Hamilton and Davies 1988). Yellow indicates the photoperiod

and water potential increases (Fig. 3). Similarly, C3 and C4 plants consume the transitory starch reserve at night, with the anticipation of replenishing this reserve with carbon fixed during the following day (Fig. 3). A consideration of the daily changes in physiology provides evidence that circadian gating of hormonal signalling couples this rhythmic regulation of reserve utilisation to rhythmic growth patterns, presumably because growth requires carbon for raw materials and water for turgor to drive cell expansion. Circadian gating of hormonal signalling also alters physiological responses to prevent temporally inappropriate responses from occurring, thus increasing the overall fitness of the plant.

In the early morning, stomata are relatively insensitive to ABA, allowing carbon uptake through open stomatal pores before the heat of the afternoon, even in moderately water-deficit stressed plants (Correia et al. 1995; Fig. 2). Stomatal opening is also favoured by high sensitivity to blue light at dawn (Fig. 2), ensuring the stomata open to maximise carbon fixation during the day (Webb 1998). Similarly, transcripts encoding proteins involved in photosynthesis are particularly sensitive to light in the morning (Harmer et al. 2000). As the day proceeds, water is lost by transpiration through the open stomata and leaf water potential decreases (Hamilton and Davies 1988; Fig. 3). This rhythmic loss of water during the day might explain the accumulation of ABA-upregulated and stress-related genes during the day (Harmer et al. 2000; Kreps et al. 2002; Mizuno and Yamashino 2008). In the early

afternoon, guard cells become more sensitive to ABA and, if there is water-deficit stress, stomatal aperture is reduced, thereby decreasing transpiration at a time when the air temperature is likely to be highest (Figs. 2 and 3).

At night, the stomata close, water reserves are replenished through the roots and the plant becomes a net consumer of carbon (Figs. 2 and 3). Transitory starch, produced from sucrose during the day and stored in the leaves, is metabolised at night to support sucrose synthesis and metabolism (Fig. 3; Smith and Stitt 2007). The plant metabolises carbon to a set point at the anticipated end of the night, ensuring that starch reserves last the entire night but are also completely consumed. If the night is artificially extended, the carbon becomes depleted before the end of the night and growth is severely inhibited (Fig. 3; Gibon et al. 2004). Once entrained to the long night, the rate of starch production is increased during the day and the rate of consumption at night is decreased (Gibon et al. 2004; Fig. 3). This leads to a reduction in growth rate to accommodate the decrease in metabolites available for growth and prevents complete inhibition of growth (Smith and Stitt 2007). Entrainment to the long night is thought to occur through a combination of signalling networks involving sugar signalling and sensing, light and the clock (Smith and Stitt 2007). Sugar metabolism and signalling networks also interact with the ABA and ethylene signalling networks (León and Sheen 2003) and the auxin and cytokinin signalling networks (Moore et al. 2003; Rolland et al. 2006).

Towards the end of the night *A. thaliana* hypocotyl growth rate is maximal (Nozue et al. 2007). Growth is controlled by a complex interplay of multiple signalling networks through the coincidence of internal circadian and external light and dark signals as demonstrated by the rephasing of the maximal period of hypocotyl expansion from subjective dusk in LL, to around dawn in LD (Nozue et al. 2007). A model describing the mechanisms for the rhythmic regulation of growth has been proposed (Nozue et al. 2007). In this model, the transcription factors PIF4 (PHYTOCHROME-INTERACTING FACTOR4) and PIF5 promote growth. *PIF4* and *PIF5* transcripts are under circadian control with transcript abundance being minimal in the middle of the night and the PIF4 and PIF5 proteins are degraded in the light. Thus, two rhythmic behaviours conspire to result in maximal growth rates around dawn; circadian control of *PIF4* and *PIF5* transcript abundance, and rhythmic light signals causing degradation of PIF4 and PIF5 proteins. Restricting growth to the night may have a number of advantages. Turgor, the driving force for cell expansion, is highest prior to dawn when there is minimal loss of water from the stomata. In addition, focusing growth to the end of the dark period might allow judicious use of carbon, allowing the demands of growth to be compared with other metabolic demands on transitory starch reserves.

Conclusions

Daily rhythms of light and heat input necessitate rhythms of uptake and use of both water and carbon by plants. Thus it is almost inevitable that there are rhythms in the processes associated with carbon and water balance, such as growth and stomatal movements. Evolutionary pressures that promoted these rhythms in physiology are likely also to have favoured rhythmic sensitivity to the hormones that regulate physiology. Particularly important will have been the development of mechanisms to prevent responses from occurring at the wrong time of day. For example, stomatal opening in response to nocturnal accumulation of auxin will result in loss of water with no gain in carbon fixation but, this is prevented, in part, by circadian gating of stomatal responses to auxin (Webb 1998). Thus rhythmic sensitivity to auxin and ABA are likely to be a common feature of many physiological processes. Similarly, the daily light/dark cycle imposes potential rhythmic abiotic stresses of heat, cold, photodamage and water-deficit stress. This too may have resulted in evolutionary pressure to favour particular stress responses such as ABA-signalling at the times of day when these stresses might be anticipated to be greatest. Evidence is also accumulating that hormone signals can influence clock function, indicating a network in which abiotic and circadian signalling are tightly integrated. The mechanisms by which the circadian clock gates hormonal signalling are unknown, but it is likely that an output of the clock modulates the hormonal signal transduction chain (Hotta et al. 2007). During extreme stresses, gating mechanisms are likely to be overridden since survival outweighs all other activities, but during more mild stress, the clock might act to modulate stress signalling to incorporate temporal information in to the network responding to cellular demands. This will maximise carbon uptake, growth and subsequent seed set and survival.

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