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TEMPORAL CONTROL OF PLANT–ENVIRONMENT INTERACTIONS BY THE CIRCADIAN CLOCK

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Abstract: The rotation of the earth generates dramatic daily and seasonal variation in the terrestrial environment. Plants, like other organisms, have evolved an endogenous biological oscillator to predict these daily changes in light and temperature and adjust to shifting seasonal conditions. This system must be robust to unexpected environmental challenges but can also assist plants to adjust physiology accordingly. Key features of the circadian system that contribute to achieve these are the process of entrainment, by which external cues adjust the state of the oscillator, and gating, by which the sensitivity to a stimulus is modified according to the time of day. In this way, the circadian clock is a dynamic hub which integrates physiology, metabolism, and growth in the context of a plant's current environment. We provide a comprehensive summary of the molecular composition of the core oscillator in Arabidopsis and the mechanisms of entrainment and gating. In particular, we focus on phytohormone signalling, photosynthetic metabolism, and defence responses as examples of core plant responses to environment that are integrated within the circadian system.

Keywords: circadian, abiotic, biotic, light, phytohormone, metabolism, defence, gating, entrainment, Arabidopsis

1 Introduction

Life has evolved on a rotating planet, which orbits the sun on a tilted axis. The orbit and rotation of the earth gives rise to dramatic daily and seasonal

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variation in the environment at any given fixed position on its surface. For terrestrial organisms, these environmental variations are particularly extreme. Plants, which are normally rooted in place for their life cycle, face heightened challenges. To adapt to these conditions, organisms from all three domains of life have evolved a timing mechanism to align their biology according to these predictable daily and seasonal rhythms (Edgar et al., 2012). This biological timer is a circadian clock (*circa,* about; *diem*, day), which generates rhythms of approximately 24 h to control metabolism, physiology, and behaviour. A defining feature of circadian rhythms is that they are sustained in continuous conditions, illustrative of their endogenous nature, and crucial to allow an organism to predict the external environment. Circadian clocks also adjust to changes in photoperiod, which allows plants to sense and adapt to seasonal variation.

There is circadian control of vegetative and reproductive growth, transpiration, metabolism, nutrient transport, and responses to the biotic and abiotic environment. There is probably not any aspect of plant physiology that is not affected by the circadian system. This is driven by an extensive regulatory network. Circadian clocks control at least 30% of the transcriptome in plants (Michael et al., 2008b), with additional layers of post-transcriptional, translational, and post-translational control. Circadian clocks confer a fitness advantage. Plants grow better when their endogenous rhythms are matched to the external light-dark cycle (Dodd et al., 2005).

Although the circadian system is broadly conserved among land plants, there is genetic variation both within and between species. This variation contributes to geographic adaptation in natural populations and has been selected for in numerous crops to adjust life cycle and improve yield (Bendix et al., 2015). For example, mutants in circadian clock genes in rice, wheat, and barley with phenotypes affecting photoperiodic flowering have been introduced into breeding programs (Turner et al., 2005; Beales et al., 2007; Faure et al., 2012; Campoli et al., 2013; Koo et al., 2013). Similarly, in elite cultivars of soybean (*Glycine max*) which have been artificially selected for specific growing regions, variation in circadian period correlates with latitude (Greenham et al., 2017). In *Brassica napus*, circadian period varies between different crop morphotypes. Root and leaf crops have longer period than oilseed crops, which is likely associated with vegetative versus reproductive productivity (Yarkhunova et al., 2016). The potential impact of selecting for circadian-regulated traits in agriculture and horticulture is only beginning to be realised because the circadian system integrates a wide range of important environmental cues and responses including drought and defence.

Circadian rhythms arise from a system comprised of three components: a core oscillator, which generates a self-sustaining rhythm; inputs, which provide reference information to adjust the state of the oscillator; and outputs, which are the processes controlled by the oscillator. The genetic architecture of the circadian oscillator in plants is distinctly different from

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animals, fungi, and even green algae and photosynthetic bacteria (Hurley et al., 2016). Nevertheless, there is deep conservation of the architecture of the circadian clock among land plants (Lou et al., 2012; Calixto et al., 2015; Linde et al., 2017). The core oscillator is comprised of three classes of transcriptional regulators: the REVEILLE (RVE) class of myb-like transcription factors expressed from the morning; PSEUDO-RESPONSE REGULATORs (PRRs) which generate a wave of transcriptional repression throughout the day; and the evening complex (EC), a night-active trimeric transcriptional repressor. An intricate understanding of the plant circadian system has been developed over the last two decades using the model genetic organism, *Arabidopsis thaliana*. Our knowledge of the circadian system in other plants is slowly expanding, but we focus here on the state of knowledge with respect to each of the three components of the circadian system in Arabidopsis.

2 Environmental Inputs to the Circadian System

The core oscillator is a gene regulatory network, which has evolved to generate a cycle with a near-24-h period. The oscillator is temperature compensated to maintain this period over a wide range of ambient temperatures, but this timing is plastic. For example, Aschoff's rule is a well-described phenomenon in chronobiology, whereby the pace of the oscillator increases with light quantity (Pittendrigh, 1960). Similarly, despite temperature compensation, the Arabidopsis clock does modestly accelerate with elevated temperatures (Salomé et al., 2010). A wide range of other internal stimuli, including metabolites and nutrient status, can also adjust the pace of the oscillator (Haydon et al., 2015).

The plasticity of the oscillator is crucial for entrainment, the process by which the phase and period of the oscillator is adjusted according to environmental stimuli. Entrainment ensures that endogenous rhythms of an organism can adjust to the continuously shifting photoperiod in the natural environment. Entrainment can also modify the timing of rhythmic processes controlled by the oscillator by less predictable events such as fluctuations in daylight, rainfall, or nutrient availability. These phase adjustments enable fine-tuning of rhythmic physiology and metabolism. Entrainment cues are called *zeitgebers* (timers), which include light, temperature, and sugar. The nature of a *zeitgeber* in circadian entrainment can be determined experimentally by generating a phase response curve (PRC), which quantifies the change in phase of circadian rhythms in response to a pulse of stimulus during free-running conditions. PRCs to pulses of either red or blue light induce dramatic phase delays early in the night and phase advances late in the night (Covington et al., 2001). In this way, the oscillator is adjusted to match the light-dark cycles according to shifting photoperiod. By comparison, the

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shape of a temperature PRC is different with phase delays around dawn and phase advances around dusk (Michael et al., 2003). A PRC to sucrose has a similar shape to light PRCs, but with less dramatic phase shifts, advanced around dawn, and delayed around dusk, which is consistent with a role for photosynthetic output adjusting rhythmic metabolism and physiology during the morning (Haydon et al., 2013).

Despite the well-defined importance of light for circadian entrainment, the precise mechanisms by which light signalling affects phase adjustment are not known. Functional red- and blue-light photoreceptors are required for sensitivity of the oscillator to light quantity. For example, under continuous red light, *phytochrome A (phyA)* and *phyB* mutants have lengthened circadian period under low and high light quantity, respectively, and *cryptochrome1 (cry1)* and *cry2* mutants have lengthened circadian period under blue light (Somers et al., 1998). The oscillator in a *phyABCDE* quintuple mutant is severely impaired in period adjustment to red light, with a nearly insensitive response to change in red light quantity (Hu et al., 2013). Thus, the importance of phytochromes and cryptochromes for light entrainment is clear, but precisely how they adjust the oscillator is not. Several genes in the circadian oscillator are strongly induced by light. NIGHT-LIGHT INDUCIBLE AND CLOCK REGULATED 1 (LNK1) and LNK2 are transcriptional coactivators of clock genes and *lnk1* and *lnk2* mutants have reduced sensitivity to period shortening by red or blue light, similar to photoreceptor mutants (Xie et al., 2014). Therefore, LNKs may represent a key component of light entrainment of the oscillator. Alternatively, since the inactive Pr form of PhyB has been proposed as a transcriptional repressor (Jung et al., 2016) and CRY2 binds to DNA and has been reported as a transcriptional activator (Pedmale et al., 2016; Yang et al., 2018), photoreceptors might regulate clock genes directly. Indeed, PhyB associates with promoters of light-responsive clock genes *LNK1*, *PRR9*, *PRR7, LUX ARRHYTHMO (LUX),* and *GIGANTEA (GI)* (Jung et al., 2016).

Two components of the circadian oscillator, PRR9 and PRR7, are necessary for temperature entrainment. A *prr7 prr9* double mutant cannot be robustly entrained to temperature cycles under continuous light and a PRC reveals insensitivity to phase adjustment of the oscillator to cold temperature pulses in the double mutant (Salomé and McClung, 2005). The temperature-sensing pathways for entrainment have not been defined, but it is notable that light signalling components are emerging as key factors in temperature sensing (Jung et al., 2016; Legris et al., 2016; Ma et al., 2016; Park et al., 2017). In particular, the temperature-dependent association of PhyB to clock gene promoters, including *PRR9* and *PRR7* (Jung et al., 2016) presents a potential mechanism for temperature entrainment.

The mechanism(s) by which temperature compensation of circadian rhythms is achieved are not yet clear. It is possible the robustness of circadian period across ambient temperatures is due to compensation by counteractive components of the oscillator and is thus an emergent property

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of the complexity of the clock. This is supported by the observation that high-order combinations of clock mutants have compromised circadian rhythms specifically at sub-optimal temperatures (Shalit-kaneh et al., 2018). Nevertheless, temperature compensation can be achieved with simple oscillators (Terauchi et al., 2007) and there appears to be specific roles for particular clock-associated proteins. For example, temperature compensation in Arabidopsis requires *GI* because a *gi* mutant has a dramatically short period at elevated temperature (Gould et al., 2006; Salomé et al., 2010). There is also a role for CRY proteins. Temperature compensation requires blue light because it is compromised in red light and in *cry1 cry2* mutants. A mathematical model based on transcript data across varying light and temperature accurately predicted dramatic changes in LATE ELONGATED HYPOCOTYL (LHY) abundance with temperature and mis-regulated LHY in *cry1 cry2* (Gould et al., 2013). Similarly to phytochromes, CRY1 contributes to thermomorphogenesis (Ma et al., 2016) but unlike the model of temperature-regulated dark-reversion to Pfr of PhyB (Jung et al., 2016), a mechanism for temperature sensing by cryptochromes has not been proposed.

Entrainment by sugar requires *PRR7* since *prr7* mutants are insensitive to period change by exogenous sucrose and are unable to adjust phase to pulses of sucrose. Depletion of endogenous sugars during the day by inhibition of photosynthesis increases *PRR7* transcript levels and lengthens circadian period (Haydon et al., 2013). Systematic modelling of PRCs to sucrose most accurately recapitulates the experimental PRC when sugar either activates expression of EC components or represses *PRR7* (Ohara et al., 2018). The arrhythmic phenotypes of EC mutants make it difficult to experimentally test the role of EC components in entrainment, but the modelling corroborates the severely compromised sucrose PRC observed in *prr7* mutants (Haydon et al., 2013).

The effect of sucrose on period also requires *BASIC LEUCINE ZIPPER 63 (bZIP63)*, a transcription factor which is activated by the starvation-responsive kinase Snf1-RELATED KINASE1 (SnRK1) and binds to the *PRR7* promoter (Mair et al., 2015; Frank et al., 2018). Over-expression of *KIN10*, a catalytic subunit of SnRK1 and *bzip63* mutants have attenuated sucrose PRCs, similar to *prr7* mutants (Frank et al., 2018). The precise mechanism by which energy status modulates SnRK1 activity is unresolved (Zhang et al., 2009; Emanuelle et al., 2015), although it is possible that there may be several signals. One possible mechanism involves trehalose-6-phosphate (T6P), a signalling sugar which closely tracks sucrose status in Arabidopsis (Figueroa and Lunn, 2016) and is reported to inhibit SnRK1 activity (Zhang et al., 2009; Nunes et al., 2013). Null mutants in *T6P SYNTHASE 1 (TPS1)* are embryo lethal (Eastmond et al., 2002; Schluepmann et al., 2003) but hypomorphic alleles are impaired in circadian period adjustment and entrainment by sucrose (Gómez et al., 2010; Frank et al., 2018). Thus, a

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signalling pathway for circadian entrainment from signal to oscillator has been defined (Frank et al., 2018). This process of modulating the phase of the oscillator by the status of photosynthesis, for example according to light availability, might be important to optimise rhythmic metabolism, growth, and physiology in photoautotrophs.

3 The Arabidopsis Circadian Oscillator

3.1 Transcription–translation Feedback Loops

Since genetic screens identified *timing of cab 1 (toc1)/prr1*, the first circadian clock mutant in Arabidopsis (Millar et al., 1995a), there has been enormous progress in understanding the transcriptional feedback loops that lie at the core of the circadian oscillator. The current model of the Arabidopsis oscillator is comprised of around 20 genes (Figure 1), which continues to expand as more components of regulatory complexes are revealed.

CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), LHY and *RVE8/LHY-CCA1-LIKE 5 (LCL5)* are expressed around dawn and encode myb-like factors which bind to evening elements (EE) and CCA1-binding sites (CBS), which are *cis*-regulatory sequences in gene promoters, to regulate transcription (Alabadí et al., 2001; Farinas and Mas, 2011; Rawat et al., 2011; Hsu et al., 2013). Their targets include *PRR9, PRR7, PRR5,* and *TOC1*, which are expressed during the day and evening, and genes encoding the EC during the night (Alabadí et al., 2001; Hsu et al., 2013; Adams et al., 2015; Kamioka et al., 2016). EEs and CBSs are also present in many circadian-regulated output genes. For example, CCA1 binds to promoter regions of ca. 1300 genes in the Arabidopsis genome (Nagel et al., 2015). CCA1 and LHY act to repress these clock genes by recruiting a corepressor, DE-ETIOLATED 1 (DET1) to EEs (Lau et al., 2011). Loss of this repression in mutants of *CCA1, LHY,* and *DET1* give rise to short-period phenotypes (Millar et al., 1995b; Green and Tobin, 1999). On the other hand, RVE8 and partially redundant homologues RVE4 and RVE6 accumulate during the afternoon and act as an activators of EE-containing genes by recruiting co-activators, LNK1 and LNK2 (Rugnone et al., 2013; Xie et al., 2014; Ma et al., 2018). LNKs interact with the LCL domain of RVE8 and are recruited to the promoters of *PRR5* and *TOC1*. LNKs, but not RVE8, interact with RNA Pol II and the histone chaperone FACILITATES CHROMATIN TRANSCRIPTION (FACT) complex and promote transcription initiation and elongation of *TOC1* and *PRR5* (Ma et al., 2018). Association of FACT complex to *TOC1* mRNA is rhythmic and closely matches *TOC1* mRNA abundance (Perales, 2007). Mutants in the activating components, *RVE4, RVE6, RVE8, LNK1,* or *LNK2*, have long-period phenotypes (Hsu et al., 2013; Rugnone et al., 2013). The activating and repressive myb-like factors are counteractive, which is

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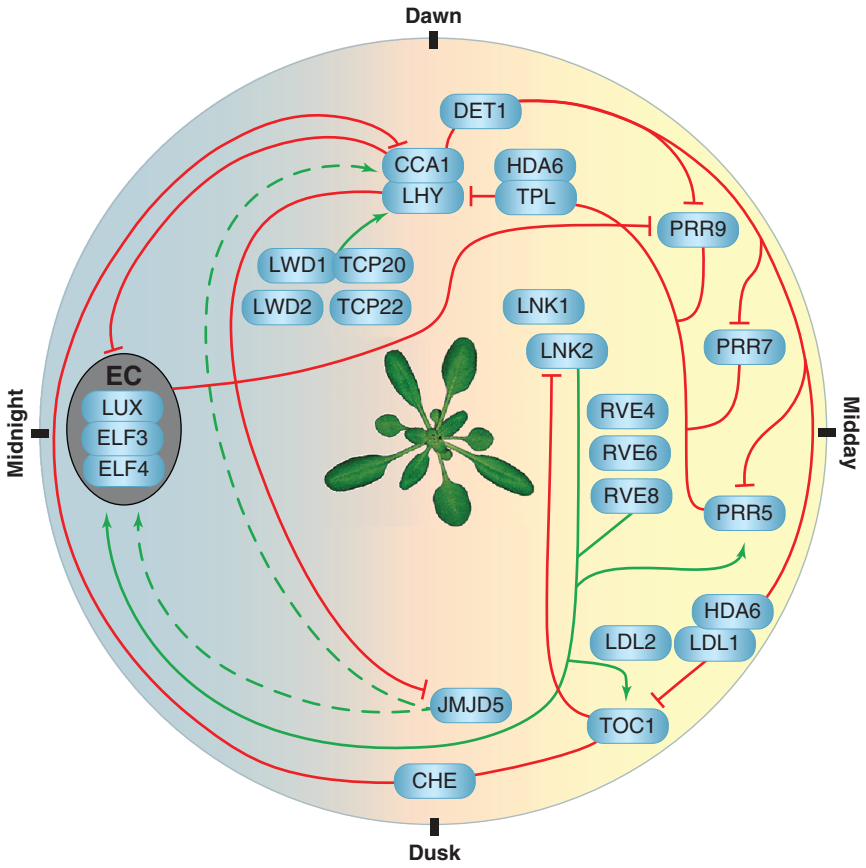

Figure 1 Transcriptional control of the Arabidopsis circadian oscillator. A summary of the current model of the components contributing to transcriptional control of the oscillator. See Section 3.1 in the main text for details. Phases of expression for each protein (blue) are represented by their position on the circle. Overlapping genes indicate protein interactions. Activating and repressive interactions are shown by green and red connections, respectively. Dashed lines indicate putative interactions.

evident in the near-wild-type period of a quintuple *cca1,lhy,rve4,6,8* mutant (Shalit-kaneh et al., 2018).

PRR9, PRR7, PRR5, and *TOC1* are expressed sequentially through the day in a wave of derepression by CCA1/LHY and activation by RVE8/6/4 (Matsushika et al., 2000). These PRR proteins bind to promoters of CCA1 and LHY to repress their expression (Nakamichi et al., 2010; Gendron et al., 2012; Huang et al., 2012). PRR9, PRR7, and PRR5 interact with TOPLESS (TPL), a member of the Groucho/Tup1 corepressor family, which recruits HISTONE DEACETYLASE 6 (HDA6) to *CCA1* and *LHY* promoters (Wang et al., 2013).

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Triple *prr9,7,5* mutants are arrhythmic (Nakamichi et al., 2005) and depletion of *TPL* lengthens period (Wang et al., 2013). TOC1 also binds to promoters and represses *CCA1/LHY* expression (Gendron et al., 2012; Huang et al., 2012), but it does not interact with TPL (Wang et al., 2013). Like other PRRs, TOC1 has general repressor activity (Gendron et al., 2012) and *toc1* mutants have a short-period phenotype (Millar et al., 1995a). TOC1 interacts with CCA1 HIKING EXPEDITION (CHE), a TCP transcription factor, which also binds to a TCP binding site (TBS) in the *CCA1* promoter and contributes to repression (Pruneda-Paz et al., 2009).

The EC, which is active during the night, is comprised of EARLY FLOWERING 3 (ELF3), ELF4, and LUX, another myb-like factor (Nusinow et al., 2011). Mutants in any one of these components are arrhythmic (Hicks et al., 1996; Doyle et al., 2002; Hazen et al., 2005). Expression of *ELF3, ELF4,* and *LUX* are repressed by CCA1/LHY and activated by RVE8/6/4 (Hazen et al., 2005; Kikis et al., 2005; Hsu et al., 2013). The EC binds to LUX-binding sites (LBS) in the promoter of *PRR9* to repress expression during the night, as well as the *LUX* promoter (Dixon et al., 2011; Helfer et al., 2011; Nusinow et al., 2011; Herrero et al., 2012). Chromatin immunoprecipitation followed by DNA sequencing (ChIP-Seq) identified hundreds of additional EC-binding sites in gene promoters, including *CCA1, PRR7,* and *GI* (Ezer et al., 2017).

In addition to derepression of morning-active genes by the PRRs and the EC, activation of *CCA1* expression involves LIGHT-REGULATED WD1 (LWD1), LWD2, and at least two TCP transcription factors (Wang et al., 2011b; Wu et al., 2008, 2016). *LWD1, LWD2, TCP20,* and *TCP22* transcript levels peak during the night (Wu et al., 2008, 2016) and mutants have short circadian period (Wang et al., 2011b; Wu et al., 2016). A *lwd1 lwd2* double mutant has a strikingly short ca. 18-h period, similar to *cca1 lhy* double mutants (Wang et al., 2011b). Genetic evidence suggests TCP20 and TCP22, and probably other Class I TCPs, activate *CCA1* expression in a LWD-dependent manner (Wu et al., 2016). LWDs lack a DNA-binding domain, but LWD1 associates with promoters of *CCA1, PRR9, PRR5,* and *TOC1* (Wang et al., 2011b; Wu et al., 2016). LWD1, and probably LWD2, physically interacts with TCP20 and TCP22 in nuclei and both associate with a TBS in the *CCA1* promoter (Wu et al., 2016).

There is also a role for chromatin remodelling in transcriptional activation of circadian oscillator genes. There are daily rhythms in histone 3 acetylation (H3ac) and trimethylation (H3K4me3) of clock genes. These histone marks are associated with transcriptional activation and peak levels coincide with maximum clock gene expression. Pharmacological inhibition of H3K4me3 or H3ac reduced amplitude and lengthened circadian period of gene expression (Malapeira et al., 2012). Conversely, inhibition of histone deacetylation increased clock gene expression amplitude and delayed phase and/or period (Perales, 2007). Inhibition of H3K4me3 with nicotinamide lead to increased association of transcriptional repressor binding to clock

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gene promoters (Malapeira et al., 2012). Thus, these histone modifications might contribute to activation of oscillator genes by modulating repressor binding.

There are distinct roles of histone demethylation in activation of morning and evening expressed clock genes. *JUMONJI DOMAIN CONTAINING 5 (JMJD5/JMJ30)* encodes a putative histone demethylase which is coexpressed with *TOC1*. Mutants have a short circadian period and might act synergistically with TOC1 in derepression of morning expressed clock genes (Jones et al., 2010; Lu et al., 2011a). CCA1 and LHY bind to the *JMJD5* promoter and repress expression (Lu et al., 2011a), suggesting a possible feedback loop. By contrast, LYSINE SPECIFIC DEMETHYLASE 1-LIKE 1 (LDL1) and LDL2, another class of histone demethylase, interact with CCA1 and LHY in a complex with a histone deacetylase HDA6. *hda6 ldl1 ldl2* mutants have increased H3ac and H3K4me at the *TOC1* promoter and reduced amplitude of *TOC1* transcript abundance (Hung et al., 2018) consistent with a role in transcriptional activation of *TOC1*.

3.2 Translational Control

In contrast to the deep knowledge of the mechanisms of transcriptional control of the circadian oscillator, our understanding of the full extent of translational regulation of circadian clocks is currently limited, particularly in plant cells. There are daily rhythms of ribosome transition into polysomes with the highest rates of translation occurring during the day (Pal et al., 2013). Microarrays of mRNA in polysomes revealed rhythms of translational state in ca. 10–50% of detected transcripts, including numerous oscillator genes. The phase of mRNA abundance and translation states were different for the majority of transcripts and the phase relationships were altered in *CCA1*-overexpressors and/or in continuous light (Missra et al., 2015). These observations suggest a role for the oscillator in regulating translation of a significant proportion of mRNAs, as well as the possibility that there is translational control of the oscillator.

Several oscillator genes have substantial phase offsets between mRNA and protein abundances, which might represent a contribution from translational control. Comparisons of mRNA and protein phase estimate a 4–8 h offset for the PRR proteins (Fujiwara et al., 2008), 3–6 h for the RVE8 (Rawat et al., 2011) and up to 8 h for ELF3 (Nusinow et al., 2011). The precise post-transcriptional mechanism(s) that give rise to these phase differences are unknown but a number of mechanisms of translational control have been linked to circadian rhythms in other organisms and might contribute to regulation of the oscillator in plants.

Codon usage is correlated with gene expression level and can affect translational efficiency. Sub-optimal codon usage in core clock genes contributes to oscillator function by affecting translation elongation rates in Drosophila,

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Neurospora, and cyanobacteria (Xu et al., 2013; Zhou et al., 2013; Fu et al., 2016). It seems likely that similar effects of codon bias could contribute to oscillator gene translation in plants.

Translation of short upstream open reading frames (uORFs) within the 5′ of mRNAs can act to repress translation of the main coding ORF. Approximately 35% of Arabidopsis transcripts are estimated to contain uORFs (Von Arnim et al., 2014). *LHY* mRNA contains several uORFs and is translationally induced by light (Kim et al., 2003a). Mutants that disrupt eukaryotic initiation factor 3 h (eIF3h) are impaired in efficient translation of several uORF containing mRNAs, including *LHY* (Kim et al., 2004). However, it is not yet clear what impact this has on oscillator function.

3.3 Post-translational Control

There are a range of post-translational mechanisms that act on proteins of the core oscillator. These affect protein maturation, subcellular localisation, transcription factor activity, and ubiquitin-mediated proteasome degradation (Figure 2). In addition, histone modifications contribute extensively to transcriptional control (see Section 3.1).

Several circadian oscillator proteins have been shown to move between the nucleus and cytoplasm including ELF3 and GI. ELF4 is required to recruit ELF3, but not LUX, to the nucleus (Herrero et al., 2012). The best-described contribution of post-transcriptional control of the oscillator involves an F-box E3 ubiquitin ligase ZEITLUPE (ZTL), which interacts with GI to control light-dependent nucleocytoplasmic partitioning and targets substrates TOC1 and PRR5 for degradation.

ZTL has properties of a blue-light photoreceptor and blue light strongly promotes its interaction with GI via a LIGHT, OXYGEN VOLTAGE (LOV) domain (Kim et al., 2007). GI is also stabilised by sucrose, which requires *ZTL,* so sugar might similarly promote the GI-ZTL interaction (Haydon et al., 2017). GI is a co-chaperone, which in cooperation with HEAT SHOCK PROTEIN 90 (HSP90), promotes ZTL maturation in the cytoplasm (Kim et al., 2011; Cha et al., 2017). After dusk, GI-ZTL dissociates, GI moves to the nucleus and mature ZTL ubiquitinates TOC1, PRR5, and CHE for degradation (Más et al., 2003; Kiba et al., 2007; Fujiwara et al., 2008; Kim et al., 2013; Lee et al., 2018). In the nucleus, ELF3 promotes interaction of GI with CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), another E3 ligase which is required for rhythms of GI protein (Yu et al., 2008). ZTL is also degraded by the proteasome (Kim et al., 2003b), but the identity of the ligase is unknown. *ztl* mutants have a long circadian period (Somers et al., 2000), consistent with a role in protein turnover of oscillator proteins. This is partially redundant with related F-box proteins FLAVIN, KELCH REPEAT F-BOX 1 (FKF1), and LOV KELCH PROTEIN 2 (LKP2) because *ztl fkf1 lkp2* triple mutants have a further lengthened period (Baudry et al.,

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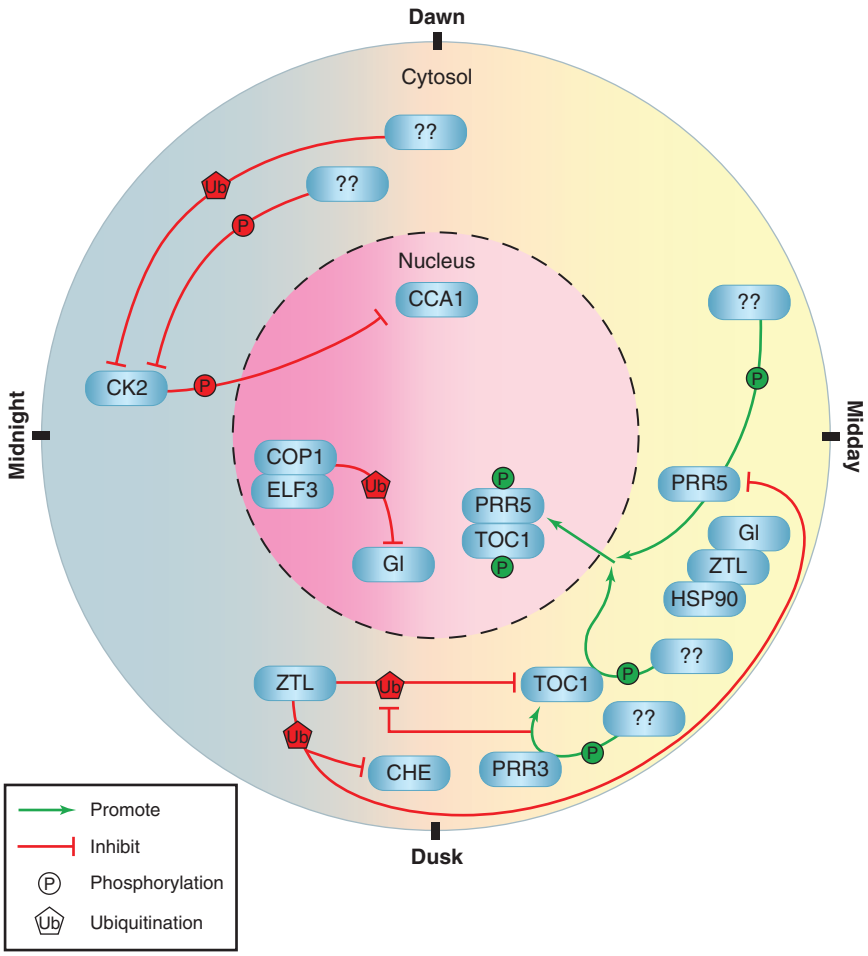

Figure 2 Post-translation control of the Arabidopsis circadian oscillator. A summary of post-translational modifications of oscillator proteins. See Section 3.2 in the main text for details. Modifications of circadian oscillator proteins (blue) that promote or inhibit protein activity are shown by green or red connections, respectively. Protein phosphorylation (circles) can affect protein interactions, nuclear localisation, or protein activity. Ubiquitination (pentagons) precedes protein degradation. Known modifications directed by unknown proteins are shown. Phases of events and protein localisation are represented by their position on the circle.

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2010). Conversely, mutants in *UBIQUITIN SPECIFIC PROTEASE 12 (UBP12)* have a short period (Cui et al., 2013) although the substrates are unknown.

The ZTL–HSP90 complex also contributes to thermal stability of the circadian clock. Warm temperature promoted the ZTL–HSP90 interaction and the complex associates with insoluble protein aggregates to degrade those damaged proteins through the ubiquitin–proteasome pathway. *ztl* and *HSP90* RNAi seedlings are hypersusceptible to heat stress with respect to growth and robustness of transcriptional circadian rhythms (Gil et al., 2017). Thus, this role for ZTL demonstrates another example of converging light and temperature signals in plants.

In cyanobacteria, phosphorylation state of the KaiC protein drives a self-sustaining post-translational oscillator (Nakajima et al., 2005; Rust et al., 2007). In Arabidopsis, there are rhythms of phosphorylation state of PRR proteins although the specific kinases are unknown (Fujiwara et al., 2008). PRR5 interacts with TOC1 and promotes its phosphorylation and nuclear localisation (Wang et al., 2010). Phosphorylated TOC1 and PRR5 more readily interact with ZTL and are targeted for degradation. Phosphorylation of PRR3 and TOC1 also promotes their interaction to prevent interaction with ZTL (Fujiwara et al., 2008), but this PRR3–TOC1 complex would be restricted to shoot vasculature (Para et al., 2007). Thus, this counteractive role of phosphorylation of TOC1 to promote both stabilisation and degradation might reflect sequential phosphorylation events controlled by distinct kinases or relate to tissue-specific features of the oscillator (see Section 3.5).

Phosphorylation of CCA1 by CASEIN KINASE 2 (CK2) contributes to alteration of promoter-binding activity (Sugano et al., 1998, 1999; Daniel et al., 2004). CK2 can phosphorylate CCA1 and inhibit its binding to promoters and thus interfere with the transcriptional repression of CCA1 target genes (Portolés and Más, 2010). CK2 is also phosphorylated preferentially in the light (Perales et al., 2006), which reduces its kinase activity and increases its susceptibility to proteasome-degradation (Hardtke et al., 2000). Thus, the effect of CK2 on CCA1 would be diminished during the day and promoted during the night. A triple mutant in CK2 catalytic subunits *ck alpha (cka1) cka2 cka3* has a long period, consistent with hypophosphorylation of CCA1 (Lu et al., 2011b). Inhibition or overexpression of CK2 impairs temperature compensation of the clock, which has been attributed to effects of warm temperature on CK2 phosphorylation and CCA1 promoter-binding (Portolés and Más, 2010).

3.4 Calcium Signalling

Calcium is a second messenger which transduces extracellular signals to regulate plant physiological and developmental processes. Environmental stimuli such as cold, salt, hormones and pathogen invasion cause Ca^{2+} influx from external and internal stores to the cytoplasm, which trigger downstream signalling (Dodd et al., 2010). There are also circadian oscillations of cytosolic

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free calcium concentrations ($[Ca^{2+}]_{cyt}$) (Johnson et al., 1995). These are driven by the core oscillator (Xu et al., 2007), but also contribute to regulate the oscillator (Martí Ruiz et al., 2018). In this way, circadian Ca^{2+} signalling could integrate environmental signals to the oscillator.

The precise channel(s) which drive circadian rhythms of cytosolic Ca^{2+} are unknown. Nicotinamide can inhibit the synthesis of cyclic adenosine diphosphate ribose (cADPR), a Ca^{2+} channel agonist in animal cells. Nicotinamide dampens circadian $[Ca^{2+}]_{cyt}$ oscillations and lengthens transcriptional circadian rhythms (Dodd et al., 2007; Hearn et al., 2018). There are circadian oscillations of cADPR which correlate with Ca^{2+} rhythms (Dodd et al., 2007) but the cADPR-activated channel(s) in plant cells are not known.

Transcript levels of oscillator genes including *CCA1* and *PRR7* are regulated by cADPR (Dodd et al., 2007) and robust rhythms of *CHE* expression can be driven by an artificially imposed circadian Ca^{2+} oscillation (Martí Ruiz et al., 2018). Input of Ca^{2+} signals to the clock requires a calcium sensor CALMODULIN-LIKE 24 (CML24) because *cml24* mutants have a long circadian period and this effect is diminished in the presence of nicotinamide or sucrose, either of which abolish circadian Ca^{2+} rhythms. Mutants in *CHE* or *TOC1* are epistatic to *cml24* (Martí Ruiz et al., 2018), pointing to a potential feedback loop between Ca^{2+} and the oscillator.

3.5 Spatial Heterogeneity of Circadian Oscillators

All plant cells contain a circadian oscillator, and circadian rhythms can be sustained in a cell-autonomous manner (Nakamichi et al., 2003). However, there is spatial heterogeneity in the genetic architecture and behaviour of the oscillator in different organs, tissues, and cell types. The circadian period of rhythmic gene expression is lengthened in roots and hypocotyls compared to leaves (James et al., 2008; Takahashi et al., 2015; Bordage et al., 2016). Circadian period is also lengthened in young leaves and the shoot apex compared to old leaves (Takahashi et al., 2015; Kim et al., 2016).

There is also spatial heterogeneity within organs. Although circadian period is lengthened in whole roots compared to shoots, precise imaging and quantification of CCA1-YFP fluorescence in nuclei of young seedlings indicates that the period of oscillations in roots tips are dramatically shorter (Gould et al., 2018). This phenomenon might also explain the observed rephasing of the oscillator in emerging lateral roots (Voss et al., 2015). There is similar heterogeneity within leaves (Wenden et al., 2012). Transcriptomes of dissected leaves and imaging of cell-type specific luciferase reporters revealed altered phase in leaf vasculature compared to whole leaves (Endo et al., 2014). Cell-type specific expression of aequorin suggests circadian oscillations of cytosolic Ca^{2+} are derived from mesophyll cells (Martí et al., 2013). Thus, there is variation in the circadian oscillator in different tissues and cell types.

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This variation could be due to tissue-specific differences of oscillator gene expression. For example, the *PRR3* promoter is strongly expressed in shoot vasculature (Para et al., 2007). Similarly, other evening-expressed clock genes cycle more robustly in vascular cells and misexpression of *CCA1*, a morning-expressed gene, in phloem companion cells damps *TOC1* expression (Endo et al., 2014). Distinct PRCs to temperature have been determined for *CATALASE3 (CAT3)* or *CAB2* rhythms, which might be due to the expression of *CAT3,* but not *CAB2*, in leaf epidermis (Michael et al., 2003). Overexpression of *CCA1* in epidermal cells, but not mesophyll or vasculature, increased sensitivity of hypocotyl elongation to warm temperature (Shimizu et al., 2015), suggesting epidermal cells are required for temperature sensing and/or growth associated with thermomorphogenesis.

Despite the spatial heterogeneity and cell autonomy of oscillators in plant cells, there is clear evidence of coupling between cells. Spatial waves of circadian gene expression have been observed in mature leaves (Wenden et al., 2012) and roots and shoots of young seedlings (Gould et al., 2018), which implies localised coupling between cells. Coupling of cellular oscillators would aid a plant to coordinate effective circadian-mediated environmental responses. At least two waves can be detected in young seedlings, each originating at the shoot and root apex, implying a hierarchy of cell type-specific oscillators (Gould et al., 2018). Indeed, circadian rhythms in roots of decapitated plants are specifically compromised in continuous light, suggesting a role for a shoot-derived signal (Bordage et al., 2016). Ablation of shoot apices led to advanced phase in leaves and damped rhythms in roots, whereas removal of cotyledons or leaves had no effect. Micrografting of shoot apices between genotypes demonstrated that circadian rhythms can be largely restored in both roots and shoots by a wild-type shoot apex on a mutant plant (Takahashi et al., 2015). The mechanisms by which neighbouring cells are coupled, or the signals derived from shoot or root apices, are yet to be defined.

4 Outputs of the Plant Circadian System

Since the core oscillator is comprised of a network of rhythmically expressed transcriptional regulators, it is straightforward to understand how the circadian system drives rhythmic physiological and developmental processes. ChIP-seq experiments have revealed the large number of direct promoter targets of oscillator proteins in the Arabidopsis genome. Over 1000 promoters are bound by each of CCA1, PRR7, or PRR5, about 150 by PRR9, over 700 by TOC1 or LHY and nearly 900 by LUX (Huang et al., 2012; Nakamichi et al., 2012; Liu et al., 2013, 2016; Nagel et al., 2015; Ezer et al., 2017; Adams et al., 2018). There are significant overlaps between target genes but also specific

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targets for each oscillator component, which would be required to generate specific phases of expression of target genes. For example, pairwise comparisons show 15–50% overlap between PRR protein targets (Liu et al., 2016) and about one-third of CCA1 targets are also bound by LHY (Adams et al., 2018). In addition to direct targets, these circadian regulators will feed into transcription factor hierarchies that have been shown to be important in environmental responses (Song et al., 2016). Thus, the basis for circadian rhythms of over a third of the transcriptome becomes evident. Furthermore, oscillator proteins also bind to promoters of nonrhythmic genes. For example, about one-third of CCA1 targets (Nagel et al., 2015) and nearly half of LHY targets are not rhythmically expressed in continuous light (Adams et al., 2018). This might suggest the circadian clock also contributes to regulating nonrhythmic processes. CCA1 and LHY might also regulate a number of genes that only have detectable rhythms of expression in light-dark cycles. The latter would imply that the contribution of the circadian clock to rhythmic expression in light-dark cycles has been underestimated.

4.1 Phytohormone Signalling

Phytohormones modulate most aspects of growth, development, and physiology. There is evidence the circadian clock contributes to control of biosynthesis and/or signalling of most phytohormones (Thain et al., 2004; Lee et al., 2006; Covington and Harmer, 2007; Michael et al., 2008a; Goodspeed et al., 2012). Among these, several play a central role in mediating responses to various environmental challenges including drought, cold, salinity, pathogens, and herbivory (Figure 3).

LHY functions prominently in the control of abscisic acid (ABA) signalling. There is significant enrichment of abscisic acid-responsive elements (ABRE) in promoters of genes bound by LHY but not CCA1 (Adams et al., 2018). ABA levels peak late in the day (Lee et al., 2006; Adams et al., 2018), consistent with a role of LHY as a repressor of ABA pathways. Induction of *LHY* represses ABA-related target genes and overexpression of *LHY* dramatically damps rhythmic accumulation of ABA under drought stress. However, rhythms of ABA levels were unaltered in *lhy* or *LHY* overexpressors in well-watered plants. ChIP-seq did not identify LHY bound to promoters of ABA biosynthesis genes. LHY might bind to biosynthesis gene promoters only under stress conditions or circadian rhythms of ABA might be controlled indirectly by LHY via a distinct oscillator component (Adams et al., 2018). The latter is supported by a role for TOC1 in ABA-mediated responses to drought. TOC1 binds to the promoter of a putative ABA-signalling gene, *ABA-BINDING PROTEIN/GENOMES UNCOUPLED 5 (ABAR/GUN5*), and *toc1* mutants and *TOC1* overexpressors have altered responses to ABA and drought (Legnaioli et al., 2009), but it is not known if these genotypes have altered rhythms of ABA.

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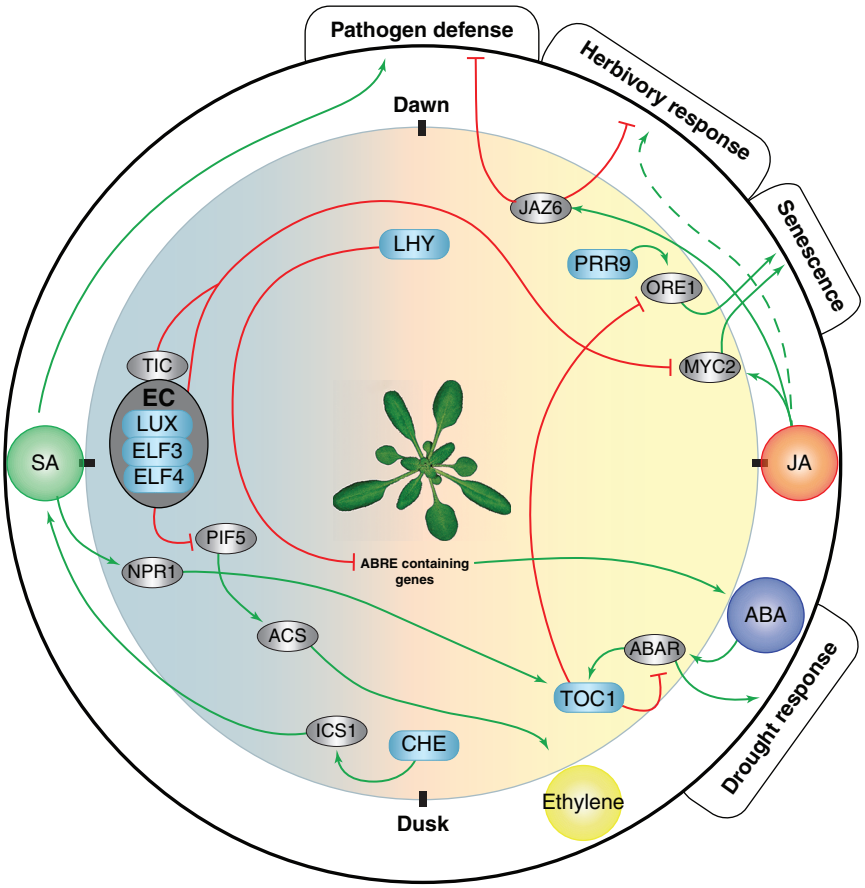

Figure 3 Phytohormones integrate environmental cues in the circadian system. The phase of circadian-regulated levels of JA, abscisic acid (ABA), ethylene, and salicylic acid (SA) are represented by their position on the perimeter of the circle. The defined interactions between these phytohormones, circadian oscillator components (blue), other genes and proteins (grey), and plant responses to environment are shown. Green and red connections represent positive and negative interactions, respectively.

There are circadian rhythms of two defence hormones, jasmonic acid (JA) and salicylic acid (SA), which are antiphased and peak during the day and night, respectively. Circadian rhythms of defence responses are important for resistance to herbivory because Arabidopsis seedlings that lack a functional oscillator, or wild-type seedlings that are grown antiphased to an herbivorous caterpillar, have reduced herbivory resistance. This is likely to require rhythmic JA, which triggers plant responses to wounding because mutants in either JA biosynthesis or JA signalling lack enhanced herbivory

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resistance conferred by resonant circadian rhythms (Goodspeed et al., 2012). Similarly, there is temporal variation in susceptibility of Arabidopsis to the fungal pathogen *Botrytis cinerea,* which is lowest during the morning. This effect is diminished in arrhythmic *elf3* mutants or in mutants of *JA ZIM 6 (JAZ6)*, encoding a transcriptional repressor of JA-mediated defence responses (Ingle et al., 2015).

Circadian rhythms of SA are regulated by CHE (Zheng et al., 2015). CHE binds to the promoter of *ISOCHORISMATE SYNTHASE 1 (ICS1)* encoding a key enzyme in SA biosynthesis. In *che* mutants, circadian rhythms of SA levels were damped but also systemic induction of SA and pathogen-triggered defence responses were diminished revealing a crucial role for the circadian oscillator in regulating SA-mediated defence.

Exogenous application of SA can alter expression of oscillator genes. Long-term application of SA increased clock gene expression, whereas short-term application reduced amplitude and delayed phase (Zhou et al., 2015; Li et al., 2018). The latter effect was phenocopied by localised *Pseudomonas syringae* infection, giving rise to systemic damping and period delay in noninfected tissues (Li et al., 2018), possibly representing a SA-mediated effect of pathogen infection on the circadian oscillator. NONEXPRESSOR OF PATHOGENESIS-RELATED 1 (NPR1) is a transcriptional activator of SA-mediated immune responses and of *TOC1* (Zhou et al., 2015), revealing at least one potential mechanism by which SA adjusts the oscillator.

Leaf senescence can be triggered by both developmental and environmental cues and can be activated by JA via MYCs, a subgroup of bHLH transcription factors. The EC acts to inhibit JA-induced leaf senescence by directly binding to the *MYC2* promoter to repress expression. Mutants in EC genes have accelerated JA-mediated senescence, which is suppressed in *myc2* mutants, as well as precocious leaf senescence phenotypes (Zhang et al., 2017). TIME FOR COFFEE (TIC), which interacts with the EC (Huang et al., 2016), also contributes to regulate MYC2. TIC interacts with MYC2 and promotes its degradation, affecting circadian rhythms of MYC2 expression and plant responses to JA (Shin et al., 2012).

PRR9 also contributes to age- and dark-induced leaf senescence and directly regulates *ORESARA1 (ORE1),* a positive regulator of ageing. *prr9* mutants have delayed senescence phenotypes (Kim et al., 2018). It is not clear if or how this pathway involves phytohormones, but *ORE1* is also regulated by ethylene signalling (Kim et al., 2009). There are circadian rhythms of ethylene emission in Arabidopsis, with levels peaking towards dusk (Thain et al., 2004). These are likely driven by direct PIF-mediated repression of *AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) SYNTHASE (ACS)* genes during the night (Song et al., 2018a). Exogenous application of ACC, an ethylene precursor, shortens circadian period and *ethylene insensitive 3 (ein3)* mutants have lengthened period (Haydon et al., 2017). Since circadian period shortens with leaf age (Kim et al., 2016), there

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might also be a role for circadian-mediated ethylene signalling in ageing and senescence.

4.2 Rhythmic Metabolism

As photoautotrophs, plant metabolism must be tightly coordinated with the light-dark cycle. In this way, plant metabolism is inextricably linked to the environment. This is highlighted by the contribution of photosynthetic metabolism to circadian entrainment (Haydon et al., 2013; Frank et al., 2018). There is circadian regulation of net CO_2 assimilation and stomatal conductance in Arabidopsis (Dodd et al., 2005). This is, in part, driven by circadian rhythms of photosynthesis-associated transcripts, which are upregulated before dawn and peak during the middle of the day (Harmer et al., 2000). Photosynthetic genes are direct targets of circadian oscillator proteins. Based on ChIP-seq data, there is enrichment of photosynthesis-related genes specifically among targets of morning expressed components CCA1, PRR9 and PRR7, and the EC (Nagel et al., 2015; Liu et al., 2016; Ezer et al., 2017). Consistent with this, comprehensive metabolite profiling in circadian clock mutants revealed greater changes in primary metabolites in *cca1 lhy*, *prr7 prr9* and *elf3* mutants compared to *toc1* or *gi* (Flis et al., 2018). Furthermore, the contributions of CCA1, PRRs and the EC to regulation of rhythmic metabolism are also distinct from each other because the metabolic profiles of the mutants are starkly different, despite similar circadian phenotypes (Nakamichi et al., 2009; Flis et al., 2018). Thus, the role of the circadian oscillator in controlling rhythmic metabolism is complex and challenging to decipher.

An elegant example of circadian regulation of metabolism is that of starch metabolism. In Arabidopsis, starch is synthesised from assimilated carbon and accumulates approximately linearly during the day. At night, starch is degraded to sustain metabolism and growth at a rate that will utilise almost all of the starch by dawn (Gibon et al., 2004; Lu et al., 2005). This pattern is maintained across a range of photoperiods, even in the event of an unpredictable early onset of dusk (Graf et al., 2010; Sulpice et al., 2014). In this way, the control of starch biosynthesis and degradation are critical to ensure that metabolic rhythms are matched to the rhythmic environment. The circadian system controls the timing of starch exhaustion because patterns of starch metabolism match the period of circadian clock mutants (Graf et al., 2010; Flis et al., 2018). However, precisely how this is achieved is unknown.

Mathematical modelling has proposed two distinct hypotheses for control of rhythmic starch metabolism. One of these models proposes two chloroplast-localised molecules: *S*, which measures the amount of starch; and *T*, which measures the time to dawn (Scialdone et al., 2013). The model supposes that these molecules interact at the surface of starch granules and approximate a division computation to calculate the rate of starch

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degradation required to nearly exhaust starch around dawn. The model accurately predicts a range of experimental data and a possible mechanism for *S* has been proposed based on phosphorylation state of starch granules. However, there is currently no candidate for *T* and so the model cannot be fully tested.

An alternative model is based on homeostatic maintenance of sucrose levels and integrates the effect of sucrose to adjust the phase of the circadian oscillator (Seki et al., 2017). This model suggests that circadian phase adjustment is not required to alter rates of starch degradation in response to an unexpected early night, because this is determined by sucrose demand. However, the model predicted that circadian phase adjustment is required to adjust rates of starch accumulation during the day according to photoperiod. Consistent with the model's predictions a *prr7* mutant, which is unable to adjust phase according to sucrose, over-accumulates starch during the day. Since circadian phase adjustment to sucrose depends on a T6P-dependent pathway (Frank et al., 2018), which correlates closely with sucrose concentration (Figueroa and Lunn, 2016) and regulates starch metabolism (Martins et al., 2013), a possible mechanism for integrating these signals into the model emerges. Nevertheless, the precise mechanism by which starch metabolism is controlled by the clock is not yet defined and could involve either or both of the proposed models.

Rhythmic metabolism is associated with cycles of redox states and production of reactive oxygen species (ROS). In plants, these are tightly associated with the availability of light to drive photosynthetic metabolism. There are circadian rhythms of H_2O_2 levels in Arabidopsis, which peak during the day and are phased similarly with catalase activity (Lai et al., 2012). There are also circadian rhythms of the reduction–oxidation coenzymes NADPH and $NADP^+$, which are antiphased (Zhou et al., 2015), and of oxidation state of peroxiredoxins (PRX) (Edgar et al., 2012). Circadian rhythms of PRX oxidation are conserved across all kingdoms of life (Edgar et al., 2012). These can be sustained in the absence of transcription and translation (O'Neill et al., 2011), which supports an intriguing hypothesis that a metabolic oscillator might have established the basis for evolution of circadian oscillators.

The oscillator directly regulates ROS pathways and ROS can affect the state of the oscillator. *CAT* genes were among the earliest transcriptional reporters for circadian rhythms in Arabidopsis (Zhong et al., 1994) and are directly regulated by the oscillator (Michael and Mcclung, 2002). Furthermore, CCA1 binds to promoters of ROS-regulated genes and overexpression of *CCA1* attenuates their transcriptional response to superoxide production and suppresses H_2O_2 accumulation (Lai et al., 2012). Transient application of H_2O_2 can delay phase (Li et al., 2018) suggesting that ROS signals might also regulate the oscillator. The effects of the immune signal, SA, on the oscillator have also been attributed to redox balance and ROS signalling (Zhou et al., 2015; Li et al., 2018). Application of SA disrupts $NADPH/NADP^+$ balance

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(Zhou et al., 2015) but is also known to trigger extracellular ROS production by RESPIRATORY BURST OXIDASE HOMOLOG (RBOH) proteins. The effects of SA on the phase of the oscillator were absent in an *rbohd* mutant (Li et al., 2018) suggesting a contribution of extracellular ROS production on circadian rhythms. Redox and ROS signals could, therefore, provide additional mechanisms by which metabolism adjusts the circadian system. However, since these signals can also be derived from various environmental challenges, they are likely involved in a wider range of circadian-mediated responses.

4.3 Gating of Environmental Responses by the Circadian Clock

The benefit of the circadian network is not only to regulate the timing of rhythmic outputs but also to control responsiveness of circadian outputs to stimuli according to the time of day. This important phenomenon is termed gating. The simplest mechanism by which gating can be controlled is through rhythmic activity of a repressor or activator. Rhythms of activity can be controlled by light or temperature, but circadian gating must be controlled by the oscillator and, by definition, must persist in constant conditions. Examples of stimuli that are gated by the clock are light, sugar, phytohormones, cold, and pathogens. In many cases, circadian gating is likely achieved by direct regulation by the oscillator because core clock genes bind to the promoters of thousands of genes in the Arabidopsis genome.

CAB2 expression is induced by pulses of light in continuous dark and responsiveness is gated, so that the effect is limited during subjective night and maximal during the subjective day (Millar and Kay, 1996). This circadian control might be important to limit the response of the oscillator to false light cues, such as moonlight. Gating of this acute response to light requires ELF3 (McWatters et al., 2000). Induction of *CAB2* by light in etiolated *elf3* mutants and *ELF3* overexpressors is enhanced and suppressed, respectively, suggesting ELF3 represses this acute response to light during the night. These genotypes are also compromised in phase resetting as shown by aberrant PRCs to red and blue light, particularly with respect to phase advances during the subjective night (Covington et al., 2001). Thus, this repressive role of ELF3 in light signalling likely contributes to entrainment.

There is also circadian gating of phytochrome-mediated shade responses in Arabidopsis. Induction of *PHYTOCHROME INTERACTING FACTOR (PIF) 3-LIKE 1 (PIL1)* expression and hypocotyl elongation by pulses of far-red light are greatest towards dusk (Salter et al., 2003). PIL1 interacts with TOC1 and gating is severely compromised in a *toc1* mutant (Makino et al., 2002; Salter et al., 2003).

Hypocotyl growth is promoted by temperature, a process called thermomorphogenesis. EC components have been identified as repressors of this

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PIF-mediated process (Box et al., 2015; Raschke et al., 2015; Jung et al., 2016). But thermomorphogenesis is also gated by the clock through TOC1 and PRR5 (Zhu et al., 2016). TOC1 and PIF4 interact and bind to an overlapping set of promoters, including a number of confirmed thermoresponsive genes. These target genes are least responsive to pulses of warm temperature in the evening when TOC1 is most highly expressed and they are derepressed in *toc1* and *toc1 prr5* mutants. Thus, TOC1 likely interacts with PIF4 to inhibit activation of target genes in the evening to gate thermoresponsive growth.

There is circadian gating of responses to phytohormones, including ABA (Legnaioli et al., 2009). Exogenous ABA induces *TOC1* expression in the evening and this requires ABAR. Since TOC1 directly regulates *ABAR*, this suggests a feedback loop. Stomatal closure in *TOC1 RNAi* plants is hyperresponsive to ABA and these lines are tolerant to dehydration, which is consistent with gating of ABA responses late in the day by TOC1.

There is circadian gating of defence responses of Arabidopsis to bacterial and fungal pathogens. Susceptibility of Arabidopsis to *P. syringae*, *B. cinerea*, or *Hyaloperonospora arabidopsidis* (downy mildew) is greater at subjective dusk than subjective dawn (Bhardwaj et al., 2011; Wang et al., 2011a; Ingle et al., 2015). Gating of immune responses to downy mildew requires CCA1. Promoters of transcripts regulated by downy mildew infection are enriched for EEs and mutants in *CCA1*, but not *LHY*, have reduced susceptibility to infection at dawn but not at dusk (Wang et al., 2011a). Gating of susceptibility to *B. cinerea* might depend directly on rhythmic JA signals because temporal variation is diminished in a *jaz6* mutant (Ingle et al., 2015).

5 Conclusion

The circadian system is complex. This complexity is crucial to integrate environmental cues into coherent physiological and developmental responses that enhance fitness. Exponential progress is being made to understand the mechanisms by which environmental signals are incorporated into the circadian system. Yet there are many questions about how diverse signals converge on the oscillator and are ultimately decoded. This is particularly important as we transfer experimental systems into the context of real environments rather than controlled laboratory conditions (Annunziata et al., 2017; Song et al., 2018b). Finally, although circadian traits have been frequently selected for (or against) in domestication and breeding of many of our staple crops (Bendix et al., 2015), there is an urgent need to accelerate the transfer of our deepening knowledge of the circadian system into the field.

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