Molecular Plant
Review Article

CellPress
PARTNER JOURNAL

Light Perception: A Matter of Time

Sabrina E. Sanchez, Matias L. Rugnone and Steve A. Kay*
Department of Neurology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
*Correspondence: Steve A. Kay (stevekay@usc.edu)
https://doi.org/10.1016/j.molp.2020.02.006

ABSTRACT

Optimizing the perception of external cues and regulating physiology accordingly help plants to cope with the constantly changing environmental conditions to which they are exposed. An array of photoreceptors and intricate signaling pathways allow plants to convey the surrounding light information and synchronize an endogenous timekeeping system known as the circadian clock. This biological clock integrates multiple cues to modulate a myriad of downstream responses, timing them to occur at the best moment of the day and the year. Notably, the mechanism underlying entrainment of the light-mediated clock is not clear. This review addresses known interactions between the light-signaling and circadian-clock networks, focusing on the role of light in clock entrainment and known molecular players in this process.

Key words: circadian clock, light signaling, *Arabidopsis thaliana*, light entrainment, photoreceptors, signal integration

Sanchez S.E., Rugnone M.L., and Kay S.A. (2020). Light Perception: A Matter of Time. Mol. Plant. **13**, 363–385.

INTRODUCTION

Plants adjust their growth, physiology, and developmental transitions to the ever-changing environmental conditions to which they are exposed. The circadian clock, an internal timekeeping mechanism, helps to integrate endogenous and external cues to ensure that coordinate growth and developmental processes occur at the right time of the day and year. On a rotating planet, light/dark and temperature cycles are highly dynamic, yet predictable, and play crucial roles in setting the endogenous clock on time through a process called entrainment. It has been shown that the resonance between the internal oscillator and the external conditions increases the fitness of *Arabidopsis thaliana* (Michael et al., 2003; Dodd et al., 2005).

Important features of circadian systems include their ability to be dynamically adjusted and to exhibit robustness toward non-relevant perturbations. Although these two characteristics might seem contradictory, they set the basis for the effective performance of an essential molecular network that influences biological rhythms. For example, dynamic adjustment of gene expression to phase protein activity at different times depending on day length is essential for flowering to occur in the correct season (Song et al., 2015). However, being insensitive to small and random changes in light or temperature protects the system from wrongly setting the time (Gil and Park, 2019). To accomplish this set of complex and comprehensive responses, multiple levels of signal integration are exhibited and numerous pathways converge on particular molecules, such as transcription factors. This multilayer control mechanism also allows plants to achieve perceptual disambiguation, which occurs when a single signal does not provide enough information to univocally specify the external condition (e.g., the same day length can occur in different seasons) and another signal is necessary to resolve the ambiguity (e.g., the memory of winter temperatures complements day-length information to define the season) (Casal and Qüesta, 2018).

To ensure synchronicity with environmental conditions, plants must constantly reset; sensing and integrating external cues is fundamental to this process. This review focuses on our current understanding of how light information is integrated into the circadian system of *A. thaliana*, and the phenotypic consequences of disruptions in the interaction between light signaling and the clock network. We also briefly discuss the crosstalk with other signaling pathways, how the molecular clockwork feeds back into light perception, and the lack of a comprehensive model to address the complex molecular output of the circadian system.

THE *ARABIDOPSIS* CORE CLOCK

The core of the biological clock is composed of a set of transcriptional-translational feedback loops (TTFL) (Huang and Nusinow, 2016; McClung, 2019), which are modulated by other cellular mechanisms including post-transcriptional regulation, and post-translational and chromatin modifications (Nohales and Kay, 2016; Mateos et al., 2018; Yang et al., 2018; McClung, 2019). In brief, these TTFL start at dawn with the induction of expression of *CCA1* (*CIRCADIAN CLOCK ASSOCIATED 1*) and *LHY* (*LATE ELONGATED HYPOCOTYL*), which encode for MYB-like transcription factors (Schaffer

Published by the Molecular Plant Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., on behalf of CSPB and IPPE, CAS.

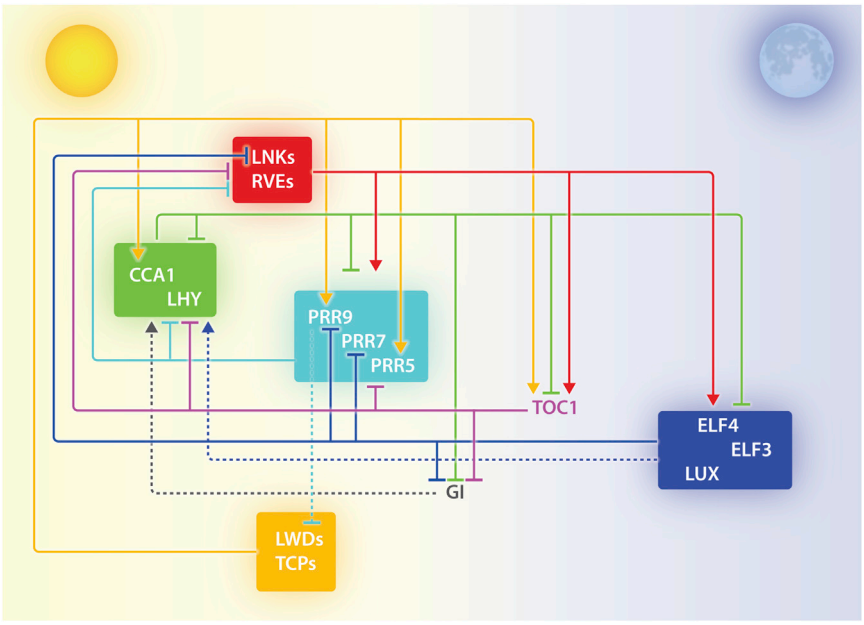

Figure 1. Simplified Representation of the Molecular Network Underlying the Circadian Clock of *Arabidopsis thaliana*.
Clock components are shown from left to right, according their time of expression throughout the day. Functional groups are enclosed in boxes. Lines with blunt ends and arrows indicate repression and activation, respectively. Broken lines represent regulatory steps not proven to be direct.

et al., 1998; Wang and Tobin, 1998). The molecular components of the biological oscillator are then expressed in temporal waves. The *PRR* (*PSEUDO-RESPONSE REGULATOR*) family of genes is expressed sequentially: the *PRR9* transcript peaks after *CCA1/LHY*, followed by *PRR7*, *PRR5*, *PRR3*, and *PRR1* (also known as *TOC1* [*TIMING OF CAB EXPRESSION 1*]) (Makino et al., 2001; Mizuno and Nakamichi, 2005). Finally, a rise in mRNA levels is observed for the evening/night players of the clock: *GI* (*GIGANTEA*), *ELF3* (*EARLY FLOWERING 3*), *ELF4*, and *LUX* (*LUX ARRHYTHMO*). The proteins encoded by *ELF3*, *ELF4*, and *LUX* interact to give rise to a transcriptional regulatory complex known as the evening complex (EC) (Nusinow et al., 2011). Each of these molecules represses the expression of many other core-clock components (Figure 1). CCA1 and LHY form homo- and heterodimers, which bind a promoter motif called the evening element to repress the expression of evening-phased genes, including *GI* and the members of the EC (Harmer et al., 2000; Lu et al., 2009, 2012; Yakir et al., 2009; Nagel et al., 2015; Kamioka et al., 2016). CCA1/LHY also negatively regulate their own expression as well as that of the *PRR* family members (Schaffer et al., 1998; Wang and Tobin, 1998; Alabadí et al., 2001; Adams et al., 2015). In turn, *CCA1/LHY* transcription can be repressed by PRR9/7/5/1 as well as the EC, closing the central negative feedback loop of the clock (Helfer et al., 2011; Huang et al., 2012; Adams et al., 2015).

The positive arms of the clock are represented by three groups of proteins: LWD1 (LIGHT-REGULATED WD 1) and LWD2 in the morning; and LNK1 to LNK4 (NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED 1) together with RVE4 (REVEILLE 4), RVE6, and RVE8 acting at midday (Wang et al., 2011; Hsu et al., 2013; Rugnone et al., 2013). LWD1 is recruited to the DNA by TCP20 (*TEOSINTE BRANCHED1-CYCLOIDEA-PCF 20*) and TCP22, and activates the transcription of *CCA1* (Wu et al., 2016). LWD1/LWD2 also promote the expression of *PRR9*, *PRR5*, and *TOC1* (Wang et al., 2011). RVE8, an MYB-like transcription factor, associates with LNK1 and LNK2 to bind *TOC1* and *PRR5* promoters (Xie et al., 2014; Pérez-García et al., 2015). RVE4, RVE6, and RVE8 have been shown to induce the expression of the EC components and *PRR*s (Farinas and Mas, 2011; Rawat et al., 2011; Hsu et al., 2013; Xie et al., 2014). The latter repress *RVE8* expression, and promoters of the LNK genes have been shown to be bound by LUX, closing another loop of the TTFL (Mizuno et al., 2014b; Zhang et al., 2019a).

The role of GI within the core clock seems to be different from that of other central components: it interacts with the F-box protein ZTL (ZEITLUPE) in a blue-light-enhanced manner and helps sustain and modulate ZTL rhythmic accumulation (Kim et al., 2007, 2013). In the dark, ZTL and GI dissociate and ZTL promotes TOC1 and PRR5 proteasomal degradation (Kim et al., 2007; Fujiwara et al., 2008). GI also induces the transcription of *CCA1* and *LHY* (Martin-Tryon et al., 2007). Recently, it was shown that PIFs (PHYTOCHROME-INTERACTING FACTORS) repress *CCA1* expression and that this activity is modulated at different levels by GI, suggesting that GI regulation of the *CCA1 locus* could occur, at least in part, through PIF proteins (Nohales et al., 2019). Finally, the expression of *GI* is negatively regulated by the EC, TOC1, and CCA1/LHY, thereby closing another loop (Huang et al., 2012; Mizuno et al., 2014a).

The circadian clock is known to regulate a myriad of physiological processes and developmental transitions and has been proposed to work as an integrator of endogenous and external signals (Sanchez and Kay, 2016). The known molecular pathways connecting the internal timekeeping mechanism and its outputs, as well as their respective feedback to the central oscillator, have been recently reviewed (Greenham and McClung, 2015; Sanchez and Kay, 2016; Singh and Mas, 2018; Creux and Harmer, 2019).

LIGHT SENSING AND THE CIRCADIAN CLOCK

Light is crucial for plant survival and success, not only because it is a very useful tool to determine the environment in which they are growing but also because it is their source of energy. Consequently, different and versatile signaling pathways have evolved to correctly sense and translate light information. The first step in this complex molecular cascade of events is light perception itself, achieved through a set of molecules known as photoreceptors, which convert detected photons into a biochemical signal

capable of being transduced through multiple molecular events (Schäfer and Nagy, 2006).

In *Arabidopsis* there are at least five families of photoreceptors: (1) phytochromes, (2) cryptochromes, (3) the ZTL family, (4) phototropins, and (5) UVR8 (*UV Resistance Locus 8*). All of these, except UVR8, bind a ligand termed chromophore (light-absorbing molecule). Each of these families presents a distinct wavelength absorption spectrum as well as different biochemical properties and molecular partners. Together, these photoreceptors allow plants to determine incoming irradiance, spectral composition, and light direction and duration (photoperiod): in other words, to have a thorough and timely outline of the light environment (Briggs, 2006; Schäfer and Nagy, 2006; Rizzini et al., 2011).

All families of photoreceptors (except phototropins) have been described to participate in light entrainment of the circadian clock, as well as contributing to setting its pace (Table 1) (Litthauer et al., 2015). However, it is worth noting that phytochromes, cryptochromes, and UVR8 are not considered intrinsic components of the clockwork because none (individually or as a family) are necessary for clock entrainment or for maintenance of robust circadian oscillations under free-running conditions (Devlin and Kay, 2000; Yanovsky et al., 2000b; Fankhauser and Staiger, 2002; Baudry et al., 2010; Strasser et al., 2010; Fehér et al., 2011; Hu et al., 2013).

In etiolated, temperature-entrained seedlings (i.e., those not exposed to light), the clock controls a smaller set of genes than it does in plants entrained with light/dark cycles and transferred to continuous light (Wenden et al., 2011). Because this observation was made in plants grown in the presence of exogenous sugar, it suggests that the reduction in the number of clock-controlled genes is not associated with starvation. Furthermore, it has been shown that the amplitude of mRNA rhythms of many genes, including core-clock genes, are significantly dampened under constant darkness (Millar et al., 1995; Bognár et al., 1999; Covington et al., 2001; Doyle et al., 2002). A possible explanation is that the drop in photosynthates in dark-grown plants affects transcriptional activity. However, it has been shown that the amplitude of *CCR2* and *CCA1* oscillations can be restored not only by the addition of exogenous sugar but also by the expression of a constitutively active form of phytochrome B (PhyB), a member of the phytochrome family of photoreceptors (see below) (Jones et al., 2015). This evidence suggests that light, instead of merely acting as an entrainment cue, might play an additional role through different photoreceptors to target the core-clock components and be a fundamental variable for sustaining the robustness of biological rhythms.

Additionally, it has been shown that light can acutely induce the expression of multiple core-clock genes (Nohales and Kay, 2016). For example, *CCA1* expression is induced upon red-light exposure as short as 90 s (Wang and Tobin, 1998). Etiolated seedlings irradiated with different wavelength lights for longer times ($\leq$2 h) also exhibit an acute response, inducing the transcription of *CCA1*, as well as *LHY*, *PRR9*, *LNK1*, *LNK3*, *ELF4*, and *TOC1* (Makino et al., 2001; Tepperman et al., 2001; Shikata et al., 2014). This rapid effect of light on the abundance of key clockwork components could affect its resetting and affect the regulation of expression of its downstream targets, which would ultimately induce a rearrangement of the transcriptome.

PHYTOCHROMES

Phytochromes have a wide spectrum of light-absorption response including red and far-red (FR), and to a lesser degree blue wavelengths. The apoprotein of phytochromes is synthesized in the cytoplasm and attached to a linear tetrapyrrole chromophore (phytochromobilin) to give rise to the inactive, red-light-absorbing form of phytochromes, Pr. After red-light illumination, the Pr form converts to the active, FR-light-absorbing form (Pfr) and translocates to the nucleus (Casal, 2013). These two photoconvertible states of phytochromes, and the photoequilibrium between them, are essential for regulation of numerous plant responses, including light input to the clock. In *Arabidopsis*, this family of photoreceptors has five members, PhyA through PhyE, with PhyA and PhyB playing dominant roles (Franklin and Quail, 2010; Wang and Wang, 2015). The apoprotein of each phytochrome is encoded by a different gene and each exhibits particular biochemical characteristics, as well as distinct and overlapping biological functions. For example, PhyB is the major phytochrome species in light-grown plants, whereas PhyA is highly abundant in dark-grown seedlings and is rapidly degraded upon exposure to light (Sharrock and Clack, 2002). PhyA is the only type I phytochrome (known as "light labile"), whereas the other four members of this family are type II ("light stable") (Franklin and Quail, 2010; Li et al., 2011b; Wang and Wang, 2015). PhyA is considered to be the main FR sensor and has also been shown to participate in the blue-light-signaling cascade. PhyB, together with PhyC to PhyE, are the dominant regulators of the responses to red light (Reed et al., 1994; Neff and Chory, 1998; Wang and Wang, 2015). PhyA and PhyB were the first and remain the best characterized members of this family of photoreceptors (Schäfer and Nagy, 2006). The existence of mutant alleles of these genes allowed researchers to conduct an initial phenotypic characterization and determine that both PhyA and PhyB participate in light input to the clock.

The free-running period of *phyB* mutant lines has been shown to be longer than that of wild-type plants at high-fluence red light, as well as to have an altered phase; *phyA* mutants have a lengthened period under low-fluence red or blue light (Table 1) (Devlin and Kay, 2000; Salomé et al., 2002; Somers et al., 1998). PhyA and PhyB act additively in red-light input to the clock, suggesting the presence of distinct signaling pathways (Devlin and Kay, 2000). Also, these two photoreceptors are important for proper re-entrainment of the clock. PhyA is necessary to re-entrain the oscillator in response to either a different "T cycle" (i.e., a 20-h day, being 10 h blue light/10 h darkness) or to a blue-light pulse at the end of the night, which shifts the phase of the clock. Although discrepancies were observed in the fluence rate of blue light that affects resetting of the endogenous rhythms in *phyA* mutants, its role in light input to the clock in response to blue light was consistent (Somers et al., 1998; Yanovsky et al., 2001). Moreover, it was established that PhyA is necessary for resetting of the clock mediated by FR light, not only in *Arabidopsis* but also in potato (*Solanum tuberosum*)

Genotype	Phenotype	Continuous condition	References
phyA	Long period	Red and blue light (fluence rate dependent)	Somers et al., 1998
PhyA OX[a]	Short period	Red light; darkness	Anderson et al., 1997; Kolmos et al., 2011
phyB	Long period	Red light (fluence rate dependent)	Somers et al., 1998; Hajdu et al., 2015
PhyB OX[a]	Short period	Dark; white light; red light (fluence rate dependent)	Somers et al., 1998; Hall et al., 2002; Kolmos et al., 2011; Hajdu et al., 2015
phyC	Long period	Red light	Jones et al., 2015
phyABCDE	Short period	Red light (low fluence); white light	Strasser et al., 2010; Hu et al., 2013
	Long period	Red light (high fluence)	Hu et al., 2013
cry1	Long period	Blue and white light; red light (fluence rate dependent)	Somers et al., 1998; Devlin and Kay, 2000
CRY1 OX[a]	Short period	Blue and white light	Somers et al., 1998
cry2	Short period	Blue light (low fluence rate); red light (fluence rate dependent)	Somers et al., 1998; Devlin and Kay, 2000
	Long period	Blue light (high fluence rate)	Somers et al., 1998
cry1;*cry2*	Long period	Blue light; red light (low fluence rate)	Devlin and Kay, 2000
ztl	Long period	Dark; blue and red light	Somers et al., 2004
ZTL OX[a]	Arrhythmic	Red light	Somers et al., 2004
uvr8	Long period	White light supplemented with UV-B light	Fehér et al., 2011
pif4;*pif5*	Long period	White light	Nohales et al., 2019
pif1;*pif3*;*pif4*	Long period	White light (with 2% sucrose)[b]	Shor et al., 2017
pif1;*pif4*;*pif5*	Long period	White light (with 2% sucrose)[b]	Shor et al., 2017
pif3;*pif4*;*pif5*	Long period	White light (with 2% sucrose)[b]	Shor et al., 2017
pifQ	Long period	Red and white light (high fluence rate)	Shor et al., 2017
PIF1 OX[a]	Short period	White light (with 2% sucrose)[b]	Shor et al., 2017
PIF3 OX[a]	Short period	White light (with 2% sucrose)[b]	Shor et al., 2017
PIF4 OX[a]	Short period	White light	Nohales et al., 2019
PIF5 OX[a]	Short period	White light	Shor et al., 2017; Nohales et al., 2019
cop1	Short period	Dark; white light	Millar et al., 1995; Yu et al., 2008
det1	Short period	Blue, red, and white light	Millar et al., 1995; Lau et al., 2011
hy5	Short period	Dark; blue, red, and white light	Andronis et al., 2008; Hajdu et al., 2018
hyh	Short period	Blue light	Hajdu et al., 2018
hy5;*hyh*	Short period	Dark; blue, red, and white light	Hajdu et al., 2018
fhy3	Long period/arrhythmic	Red light	Allen et al., 2006
	Short period	Blue light	Allen et al., 2006
	Short period/arrhythmic	White light	Li et al., 2011a
far1	Short/long period (assay dependent)	White light	Li et al., 2011a
hy1	Long period	Red light	Millar et al., 1995
cor27	Long period	White light	Li et al., 2016
cor28	Long period	White light	Li et al., 2016
cor27;*cor28*	Long period	White light	Li et al., 2016

Table 1. Circadian Period-Length Phenotype of Light-Signaling Mutants.

[a]*OX*, overexpressing line.

[b]Sucrose was specified as an important variable for the phenotype.

Core-clock protein	Light-signaling protein	Experimental technique	References
CCA1	PhyB	Y2H, coIP	Yeom et al., 2014
	FHY3	Y2H, coIP, LCI	Li et al., 2011a
	DET1	Y2H, *in vitro* assay, BiFC, LCI	Lau et al., 2011
	HY5	Y2H, *in vitro* assay	Andronis et al., 2008
	CSU4	Y2H, BiFC, coIP	Zhao et al., 2018
LHY	PhyB	coIP	Yeom et al., 2014
	FHY3	Y2H	Li et al., 2011a
	DET1	*in vitro* assay, LCI	Lau et al., 2011
PRR9, PRR7	PIF3	Y2H, *in vitro* assay, BiFC	Martín et al., 2018; Zhang et al., 2020
	PIF4	Y2H, *in vitro* assay	Martín et al., 2018; Zhang et al., 2020
	PIF7	*in vitro* assay	Zhang et al., 2020
PRR5	PIF3	Y2H, BiFC	Martín et al., 2018
	PIF4	Y2H, BiFC, coIP	Zhu et al., 2016; Martín et al., 2018; Zhang et al., 2020
	PIF7	*in vitro* assay, BiFC, coIP	Zhang et al., 2020
	ZTL	Y2H, *in vitro* assay, coIP,	Yasuhara et al., 2004; Kiba et al., 2007; Fujiwara et al., 2008
	FKF1	Y2H, *in vitro* assay, coIP	Baudry et al., 2010
	LKP2	Y2H, *in vitro* assay, coIP	Yasuhara et al., 2004; Baudry et al., 2010
GI	PhyB	Y2H, coIP	Yeom et al., 2014
	PIF1, PIF4	Y2H, *in vitro* assay	Nohales et al., 2019
	PIF3, PIF5	Y2H, *in vitro* assay, coIP	Nohales et al., 2019
	ZTL	Y2H, *in vitro* assay, coIP	Kim et al., 2007
	FKF1	Y2H, *in vitro* assay, coIP	Sawa et al., 2007
	LKP2	Y2H, *in vitro* assay	Kim et al., 2007
TOC1	PhyB	Y2H, coIP	Yeom et al., 2014
	PIF1 (PIL5)	Y2H	Yamashino et al., 2003
	PIF2 (PIL1)	Y2H	Makino et al., 2002
	PIF3	Y2H, BiFC, coIP	Makino et al., 2002; Soy et al., 2016
	PIF4	Y2H, coIP	Yamashino et al., 2003; Zhu et al., 2016
	PIF5 (PIL6)	Y2H	Yamashino et al., 2003; Fujimori et al., 2004
	PIF6 (PIL2)	Y2H	Yamashino et al., 2003
	PIF7	2HP, BiFC	Kidokoro et al., 2009
	ZTL	Y2H, *in vitro* assay, coIP	Mas et al., 2003; Fujiwara et al., 2008
	FKF1	Y2H, *in vitro* assay, coIP	Mas et al., 2003; Baudry et al., 2010
	LKP2	Y2H, *in vitro* assay	Mas et al., 2003; Yasuhara et al., 2004; Baudry et al., 2010
LUX	PhyB	coIP	Yeom et al., 2014
	PCH1	AP–MS	Huang et al., 2016a, 2016b
ELF4	PCH1	AP–MS	Huang et al., 2016a, 2016b

Table 2. Protein–Protein Interactions between Core-Clock and Light-Signaling Network Components.

(Continued on next page)

Core-clock protein	Light-signaling protein	Experimental technique	References
ELF3	PhyB	Y2H, *in vitro* assay, coIP, AP–MS	Liu et al., 2001; Yeom et al., 2014; Huang et al., 2016a; Kim and Somers, 2019
	PhyA, PhyC, PhyD, PhyE	AP–MS	Huang et al., 2016a
	PIF4	Y2H, BiFC,	Nieto et al., 2015
	PIF7	AP–MS	Huang et al., 2016a
	COP1	Y2H, coIP, BiFC, AP–MS	Yu et al., 2008; Huang et al., 2016a
	SPAs (SPA1 to SPA3)	AP–MS	Huang et al., 2016a
	PCH1	AP–MS	Huang et al., 2016a, 2016b
ZTL	PhyB	Y2H, *in vitro* assay	Jarillo et al., 2001
	CRY1	Y2H, *in vitro* assay	Jarillo et al., 2001
	FKF1	Y2H, coIP, FRET	Takase et al., 2011
	LKP2	Y2H	Yasuhara et al., 2004

Table 2. *Continued*

Due to its dual function, ZTL was included as a clock protein as well as a member of the light-signaling pathway. AP–MS, affinity purification and mass spectrometry; BiFC, bimolecular fluorescence complementation; coIP, co-immunoprecipitation; FRET, Förster resonance energy transfer analysis; LCI, firefly luciferase complementation imaging; Y2H, yeast two-hybrid; 2HP, two-hybrid system in protoplasts.

(Yanovsky et al., 2000a, 2001). PhyB was shown to be involved in red-light resetting in both *Arabidopsis* and tomato. This was determined by assessing the ability of modified plants (*phyB* mutant *Arabidopsis* and an underexpressor line of PhyB tomato) to adjust the phase of *CCA1*:*LUC* (*CCA1* promoter fused to the *LUCIFERASE* gene reporter) and leaf angle, respectively, after red-light illumination at the end of the night (Yanovsky et al., 2000a; Hajdu et al., 2015). It has been suggested that endogenous levels of PhyB are required and sufficient for the proper resetting of the clock mediated by red wavelengths, as PhyB-overexpressing lines do not exhibit an exacerbated response to a red-light pulse compared with wild-type plants (Hajdu et al., 2015). Nevertheless, the pace of the period correlates with phytochrome dose: *phyA* and *phyB* mutants have increased period length and *PhyA* and *PhyB* overexpressors have reduced period length (Table 1) (Somers et al., 1998; Devlin and Kay, 2000; Hall et al., 2002; Kolmos et al., 2011).

Gene expression of morning and evening core-clock components has been shown to be decreased and increased, respectively, by FR light in a PhyA-dependent manner, suggesting that this photoreceptor also has an important role in FR light input to the oscillator (Wenden et al., 2011). Moreover, the analysis of *CCA1*:*LUC* expression profile under continuous FR light on *gi*, *toc1*, and *elf4* mutants suggests that these genes contribute to the FR signaling pathway of the clock (Wenden et al., 2011).

Phytochromes C, D, and E are less abundant than PhyA and PhyB. PhyD and PhyE display a partially redundant function with PhyB (Franklin and Quail, 2010). The evidence suggests that PhyC and PhyE are not able to homodimerize (a necessary condition for signaling activity in *Arabidopsis*) but heterodimerize either with PhyB or PhyD, and are thus hypothesized to modulate the activity of the latter (Clack et al., 2009; Jones et al., 2015). Nonetheless, despite not having a prevailing role in the biological oscillator, PhyC, PhyD, and PhyE have been shown to participate in red-light input to the clock (Devlin and Kay, 2000; Jones et al., 2015).

Despite evidence for the function of phytochromes on the entrainment and fine-tuning of the circadian clock, two research groups have shown that plants lacking this whole family of photoreceptors are still able to achieve rhythmicity after light–dark entrainment and to maintain oscillations under free-running conditions, indicating that phytochromes are not an intrinsic component of the clockwork itself (Zhong et al., 1998; Strasser et al., 2010; Hu et al., 2013).

PHYTOCHROME SIGNALING

Phytochrome signaling is extremely complex and divergent, as it controls numerous cellular processes including gene expression, alternative promoter selection, post-transcriptional regulation (alternative splicing), cytosolic mRNA translation, and protein localization (Palágyi et al., 2010; Paik et al., 2012; Shikata et al., 2014; Wang and Wang, 2015; Ushijima et al., 2017). Specifically, PhyB has been shown to regulate the transcriptional activity and phase of several core-clock genes (Palágyi et al., 2010). The signaling pathway underlying that regulation and the mechanism responsible for the ability to modulate the period length remain unclear. However, many molecular interactions between central components of light-signaling cascades and the circadian clock have been identified, paving the way for unraveling the mechanisms that connect light input with the clockwork (Table 2 and Figure 2). One of the earliest interactions to be described was the association between PhyB and ELF3; based on genetic data, ELF3 was proposed to be part of PhyB signaling for at least some physiological responses such as early morphogenesis (Liu et al., 2001). By then, the relevance of *ELF3* in light signaling and in the clock network was clearly established, as the photoperiod-insensitive flowering of the *elf3-1* mutant had suggested its involvement on the circadian clock (Zagotta et al.,

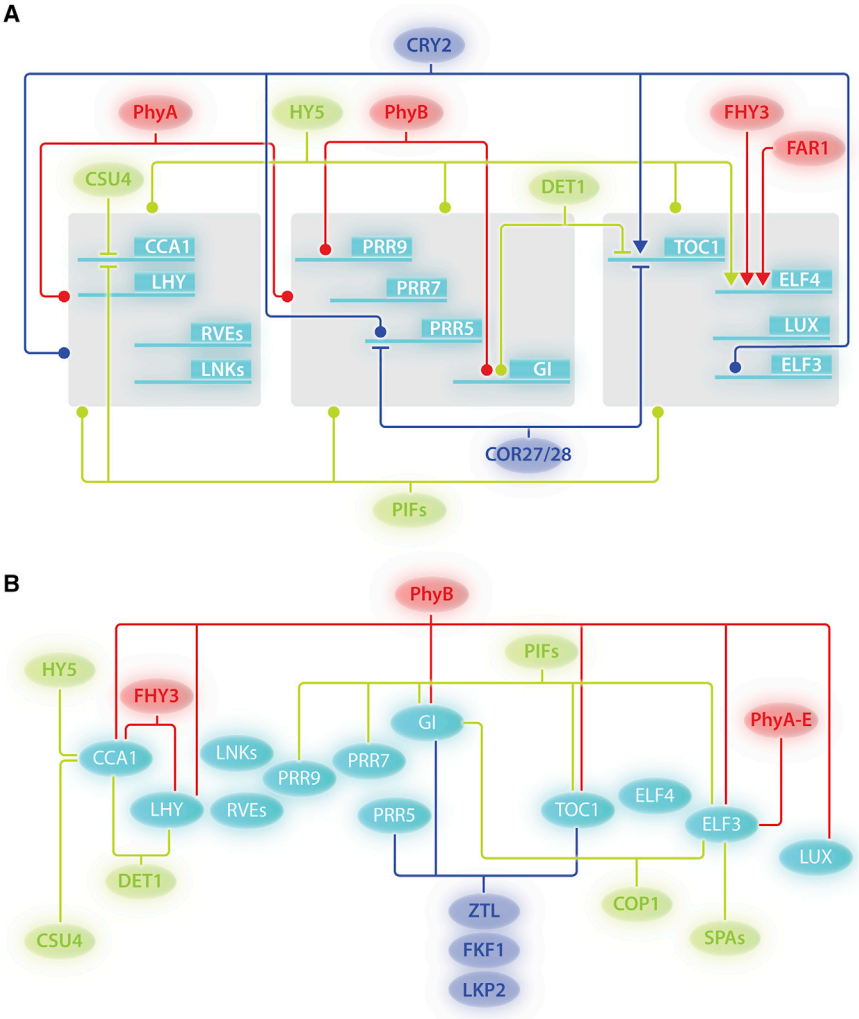

Figure 2. Regulation of Core-Clock Elements by Light-Signaling Proteins.

(A) Transcriptional regulation of core-clock genes by photoreceptors or components of light-signaling pathways. The presumed regulation is based on various sources of experimental evidence but does not include genetic data. Dot-ended lines represent protein–chromatin associations for which the biological role (i.e., transcriptional regulation) has not been described. Lines with blunt ends and arrows indicate repression and activation, respectively. Cyan lines and boxes indicate clockwork genes. Gray boxes group genes targeted by the same component.

(B) Protein–protein interactions between core-clock players and components involved in light perception or signaling. Cyan ovals indicate clockwork elements. Regulatory elements belonging to different light wavelength signaling cascades are represented with distinct colors: Red elements, red light; blue elements, blue light; green elements, regulatory players shared by different light wavelength perception pathways. Ovals represent proteins.

1996). Later, it was shown that seedlings carrying *elf3* mutant alleles are arrhythmic under constant light, but not under constant dark conditions (Hicks et al., 1996; Reed et al., 2000). Moreover, *elf3* mutants kept under constant light but with temperature cycles (i.e., temperature entrainment) partially maintain their rhythmicity, supporting the relevance of *ELF3* in light input to the clock (McWatters et al., 2000). Finally, the expression of the *CAB2* (*CHLOROPHYLL A/B-BINDING PROTEIN 2*) gene is known to be acutely induced by a pulse of light, but the magnitude of the induction depends on the time of the day at which the stimulus occurs. This process, known as gating, is controlled by the endogenous oscillator and modulated by *ELF3*; the acute response of *CAB2* expression upon light exposure becomes "time-insensitive" and is enhanced in *elf3* mutants (McWatters et al., 2000). Consistently, ELF3 was found to play a role in the resetting of the circadian clock in response to light treatment at different times of the day, and was suggested to antagonize light input during the night (Covington et al., 2001). This evidence, together with the more recent confirmation that *ELF3* is a component of the core-clock itself, positioned this gene as a strong candidate for connecting light and circadian networks (Thines and Harmon, 2010).

Further studies confirmed the PhyB–ELF3 interaction. In experiments described in a letter to the Editor, Yeom et al. (2014) used yeast two-hybrid assays to show that PhyB can interact not only with ELF3 but also with GI, TOC1, and CCA1. Moreover, using transiently transfected protoplasts and co-immunoprecipitation assays, the authors confirmed those results and showed that LUX and LHY also associate with PhyB. Interestingly, some of these interactions are light quality dependent, suggesting that PhyB binding might depend on its Pr/Pfr state, and implying a significant biological relevance: it could represent a strategy by which phytochromes convey to the clock both light/dark information and also more subtle and complex information on light status (i.e., shaded conditions or dusk, characterized by a lower red/FR light ratio). Finally, Huang et al. (2016a) conducted an unbiased experiment to determine partners of ELF3 and ELF4 *in vivo*. The authors employed transgenic lines in which the level of accumulation of the protein of interest was similar to that of the endogenous and purified protein extracts from plants under normal growth conditions. By using affinity purification followed by mass spectrometry, the researchers discovered that ELF3 associates not only with PhyB but also with the other four phytochromes and with pivotal light-signaling components, such as COP1 (CONSTITUTIVE PHOTOMORPHOGENIC 1), three members of the SPA (SUPPRESSOR OF PHYA-105) family of proteins, and PIF7 (see below). Furthermore, it was shown that ELF4 interacts with most of these proteins in an ELF3-dependent manner, reinforcing the notion that ELF3 acts as a hub to link circadian and light-signaling networks (Huang et al., 2016a). Although the biological relevance of PhyB–ELF3 interaction is not fully understood, it has been shown that PhyB stabilizes ELF3 (Nieto et al., 2015). Additionally *elf3-14*, a hypomorphic allele of *ELF3*

carrying a single amino acid substitution (A37T) within the known ELF3–PhyB interaction domain, exhibits reduced association with the photoreceptor (Kim and Somers, 2019). These data, together with the developmental phenotypes displayed by the mutant alleles of these genes, suggest that PhyB–ELF3 interaction could be at least somewhat important for PhyB-positive signaling through ELF3. The dynamic of this partnership (which has not yet been addressed) is essential for understanding the molecular mechanism of light input to the clock, and should be further studied.

PCH1 (PHOTOPERIODIC CONTROL OF HYPOCOTYL 1) directly interacts with the five members of the phytochrome family and is required for the formation of photobodies, the characteristic subnuclear foci formed by PhyB after photoactivation (Huang et al., 2016a, 2016b, 2019). Photobodies were shown to be critical for transducing light signals (Huang et al., 2019). Overexpressing lines of a constitutive active allele of PhyB maintain circadian oscillations after transfer to darkness in a wild-type background, but fail to do so in the *pch1* mutant genotype. This demonstrates that PCH1 and, likely, the formation of photobodies, are necessary for PhyB-mediated light input to the circadian oscillator (Huang et al., 2019). This finding confirms the essential role of PhyB in light input to the clock. Additionally it suggests that PhyB is not sufficient to convey light information to the clockwork and that its signaling pathway must also be functional, at least under the conditions tested (i.e., after transfer to darkness).

It is tempting to speculate that the role of phytochromes on light-signal integration into the core clock occurs through multiple mechanisms, including by targeting clockwork components directly, as well as regulating molecular hubs (e.g., COP1 and PIFs; see below), which in turn modulate transcriptional activity and protein function of essential clock players. Additionally, PhyB associates with chromatin and the bound regions exhibit a significant enrichment of G-box motifs. In particular, this photoreceptor has been shown to bind *GI* and *PRR9* promoters and has been proposed to be a transcriptional repressor (Jung et al., 2016). Although the physiological relevance of these associations has yet to be determined, they suggest that PhyB could directly regulate transcriptional activity of core-clock genes.

PhyA, despite lacking a known DNA-binding domain, also has the ability to associate with chromatin and has been found to bind many core-clock gene promoters (Figure 2). The current model proposes that PhyA interacts with its target regions through canonical transcription factors, providing a global mode of action to regulate gene transcription (Chen et al., 2014).

FHY3 (FAR-RED ELONGATED HYPOCOTYL 3) is a transcription factor that has been identified as a component of the PhyA signaling pathway, specifically in the PhyA-mediated high-irradiance response. *fhy3* mutant is hyper-responsive to red light during seedling establishment, suggesting that it might also have a role downstream of other phytochromes (Whitelam et al., 1993; Yanovsky et al., 2000c). Supporting this hypothesis, FHY3 was shown to regulate different features of the biological timekeeper, almost exclusively in a red-light-dependent manner. *fhy3* mutants exhibit an altered phase and amplitude of gene expression compared with wild-type plants, as well as defects in resetting upon red-light treatments, suggesting this gene could be involved in red-light input to the clock (Allen et al., 2006).

FAR1 (*FAR-RED IMPAIRED RESPONSE 1*), a paralog of *FHY3*, is also part of the PhyA signal transduction and participates in the regulation of seedling development (Lin et al., 2007). *FAR1* and *FHY3* are essential for the amplitude and rhythmic expression of *ELF4*, whereas *HY5* (*Long Hypocotyl 5*) and its homolog *HYH* (*HY5 HOMOLOG*), two basic leucine zipper domain (bZIP) transcription factors also involved in PhyA signaling, only modulate the amplitude of *ELF4* expression (Li et al., 2011a). Although a role for HY5 in light input to the clock has been long established, its molecular mechanism was not understood (Anderson et al., 1997). Recently, FAR1, FHY3, and HY5 have been shown to bind *ELF4* promoter *in vivo* and induce its expression. Moreover, FHY3 is able to interact with CCA1, LHY, and HY5. Thus, the proposed model suggests that FAR1, FHY3, and HY5 induce *ELF4* expression by direct association with its promoter, and the binding of CCA1 and LHY to that complex on *ELF4 locus* represses their ability to activate transcription. The activation of *ELF4* transcription, mediated by FAR1, FHY3, and HY5, contributes to the circadian profile of expression of this key component of the EC and represents a light-input pathway to the clock, as these three transcription factors are regulators of the PhyA signaling (Li et al., 2011a; Gangappa and Botto, 2016). HY5 has been found to have an additional role as part of the blue-light input to the clockwork (see details below) that could help to integrate information from multiple wavelengths.

PIF SIGNALING

PIFs are a subfamily of basic helix-loop-helix (bHLH) transcription factors, originally described as pivotal components of red/FR light signaling through characterization of their role in hypocotyl growth. For example, PIF3, the founding member of this protein family, was identified as a direct partner of PhyA and PhyB, and those interactions were shown to be necessary for signal transduction of both photoreceptors and the consequent regulation of seedling de-etiolation (Ni et al., 1998; Kim et al., 2003a; Monte et al., 2004; Castillon et al., 2007). However, it is now clear that PIFs are molecular hubs at the crossroads of multiple cellular pathways such as light (not exclusively through phytochromes) and temperature signaling, biotic and abiotic responses, hormone signaling, sugar metabolism, and the circadian clock (Leivar and Monte, 2014; Paik et al., 2017). Integration of such assorted internal and external information allows PIFs to shape plant growth and development.

Essentially, upon light exposure, activated phytochromes translocate from the cytoplasm to the nucleus where they promote PIF turnover through phosphorylation, ubiquitylation, and degradation. Hence, a reprogramming of the transcriptional landscape induces physiological and developmental changes (Leivar and Monte, 2014). Nevertheless, the current knowledge broadens that paradigm (Pham et al., 2018a). For example, PIF2 (also known as PIL1) has been shown to be stabilized by PhyB upon red-light irradiation (Luo et al., 2014). PIF7, a major regulator of

shade response, exhibits a different mode of action whereby phosphorylated PIF7 associates with photoactivated PhyB in the nucleus, and upon exposure to shade conditions (low red/FR light ratio), PIF7 becomes rapidly dephosphorylated and binds target promoters (Li et al., 2012). Notably, PIF7 activity is likely regulated by phosphorylation/dephosphorylation and subcellular localization, instead of ubiquitylation and proteasome-mediated degradation as is the case for other PIFs (Li et al., 2012; Huang et al., 2018; Pham et al., 2018a). Finally, PIFs are also known to regulate PhyB abundance, which is dependent on their interaction and imposes a mutually negative feedback loop (Leivar et al., 2008, 2012).

The convergence of core-clock components and PIFs to regulate clock outputs has been clearly established, and multiple interactions between players of those two signaling pathways have been identified (Table 2). A role for PIF4 and PIF5 linking the circadian clock and red-light signal transduction pathways was proposed early on (Yamashino et al., 2003; Fujimori et al., 2004). The TOC1–PIF3 module has been established as a diurnal growth pattern regulator, with TOC1 directly interacting with PIFs and repressing their transcriptional activity during the early night (Soy et al., 2016). Furthermore, PRR9, PRR7, and PRR5 bind to PIF3 and PIF4 and repress their transcriptional activity, and limit the time of action of PIF4 to the end of the night/pre-dawn, when they trigger hypocotyl elongation (Martín et al., 2018). PRR9, PRR7, and PRR5 also bind PIF7 (Zhang et al., 2020). Plant growth regulation mediated by PIFs is also modulated by ELF3 (which represses PIF4 protein activity upon association) and by GI–PIF interaction (which interferes with PIFs' stability and DNA-binding ability) (Nieto et al., 2015; Nohales et al., 2019). Moreover, the EC has been shown to bind to and regulate *PIF4* and *PIF5* expression, contributing to the circadian control of hypocotyl growth dynamics (Nozue et al., 2007; Nusinow et al., 2011). TOC1–PIF4 interaction has been associated with the circadian gating of thermoresponsive growth, and TOC1–PIF7 is involved in the transcriptional regulation of low-temperature stress-responsive genes (Kidokoro et al., 2009; Zhu et al., 2016).

In view of the numerous interactions between core-clock components and PIF proteins, and considering that these transcription factors bind to DNA (particularly to G-box elements), that there is an overrepresentation of G-box motifs on the promoter of clock-controlled genes (including central players of the circadian oscillator) and that PIF activity is repressed upon interaction with some key clock proteins, it is tempting to speculate that each one of these associations could feedback into the central oscillator (and other outputs). Thus, besides modulating downstream growth responses, interactions between core-clock components and PIF proteins are one more gear of the oscillator: clock components modulate PIF transcriptional activity, which affects the expression pattern of core-clock genes (Martinez-Garcia et al., 2000; Hsu et al., 2013; Zhu et al., 2016; Nohales et al., 2019). It has been shown that overexpression of PIF4 can destabilize ELF3, suggesting another possible mode of action for signaling into the clock (Nieto et al., 2015). Nevertheless, the role of PIFs in the functioning of the biological timekeeper has been elusive until recently, probably because of their partial functional redundancy and the robustness of the circadian network (Fujimori et al., 2004; Viczián et al., 2005; Nusinow et al., 2011; Nohales et al., 2019).

PIF-overexpressing lines have shown distinct clock behavior in different studies, but accumulating evidence suggests that higher levels of PIFs induce a shortening of the free-running period (Fujimori et al., 2004; Viczián et al., 2005; Shor et al., 2017; Nohales et al., 2019). In addition, the double mutant *pif4*;*pif5* has no significant period-length difference compared with the wild-type plant when *pTOC1*:*LUC* reporter is assayed, but exhibits a long period if *pCCA1*:*LUC* is assessed (Nusinow et al., 2011; Nohales et al., 2019). These discrepancies can be easily explained by multiple factors, including a more direct/indirect effect on different promoters or variations within the experimental setup (e.g., light quality and irradiation); most likely they also represent the complex effect exerted by this transcription factor family on the ticking of the molecular clock, particularly on the pace of the oscillator.

Recently, molecular evidence supporting the notion of PIFs signaling into the circadian network and functioning as important pieces of the light and metabolic input was found. PIFs were shown to bind (*in vitro* and *in vivo*) to the promoters of core-clock components (Martinez-Garcia et al., 2000; Hornitschek et al., 2012; Oh et al., 2012; Zhang et al., 2013; Pfeiffer et al., 2014; Shor et al., 2017; Nohales et al., 2019). Gene-expression analysis comparing *pifQ* (*pif1*;*pif3*;*pif4*;*pif5*) mutant and wild-type plants grown under short-day conditions, as well as transactivation assays, showed that PIFs are able to repress *CCA1* expression. In addition, assessment of *pCCA1*:*LUC* reporter activity in etiolated seedlings transferred to light revealed that wild-type plants exhibit a higher light-induced transcriptional activity than PIF-overexpressing lines but a lesser level of induction than the *pif4*;*pif5* mutant background. This, together with the hypersensitive phase-shift response to light pulses observed on the *pif4*;*pif5* mutant, establishes that PIFs have a role in light input to the clock (Nohales et al., 2019). Surprisingly, another study also reported that PIFs are involved in setting the pace of the clock, but as players of the metabolic input (Shor et al., 2017). Shor et al. (2017) determined that the long-period phenotype of the *pifQ* mutant is fluence rate dependent. This prompted the authors to question the role of PIFs in light input to the clock and to investigate their role in sugar signaling, the results of which showed the expression level of *PIF*s to be modulated by sugar. The presence of 3% sucrose enhanced PIFs' ability to bind *CCA1* and *LHY* promoters at subjective dawn, which correlated with a peak of expression of those two genes in the wild-type background and a delayed occurrence of such a peak in *pifQ* mutants. These findings led the authors to suggest that PIFs may be required for sucrose-mediated induction of *CCA1* and *LHY* expression (Shor et al., 2017). This apparently conflicting evidence could be the result of PIFs acting as hubs of different signaling pathways, as has been determined for other physiological responses (Leivar and Monte, 2014; Paik et al., 2017).

Sucrose signaling to the central oscillator depends on *PRR7*, expression of which is repressed by photosynthetically derived sugars, likely in a PIF-independent manner (Haydon et al., 2013; Shor et al., 2017). PRR7, a core-clock component and *CCA1*/*LHY* transcriptional repressor, associates with chromatin and has been shown to co-bind with PIFs to many dawn-phased genes (Liu et al., 2013, 2016b; Martín et al., 2018).

Furthermore, other PRR family members interact with PIFs and gate their activity to gene expression (Martín et al., 2018). It could be hypothesized that low fluence rates of light—and therefore low levels of sucrose—imply high expression of *PRR7*, which would translate to strong repression of *CCA1/LHY*. Under such conditions the role of PIFs on the *CCA1/LHY* repression could be masked due to the reduced levels of those transcripts. In contrast, higher-fluence rates—and higher concentration of sucrose—imply repression of *PRR7* and higher expression of *CCA1/LHY*, which sensitizes the system and exposes the proposed role of PIFs on repressing these two morning genes. Moreover, GI can hinder the binding of PIFs to chromatin (Nohales et al., 2019). It would be valuable to address whether PRR7 has the same ability, as it could also explain the increased binding of PIFs to *CCA1/LHY* promoters in the presence of sugar, a condition whereby PRR7 would be less abundant. This hypothesis locates PRR7 and PIFs as hubs for signaling integration and transduction to the circadian clock, able to modulate the expression of two pivotal core-clock genes depending on two of the most relevant environmental and internal conditions: light and sugar metabolism. Reinforcing the idea of significant crosstalk between PIFs and PRR7 on the regulation of *CCA1* expression is the fact that the sucrose-mediated morning induction of *CCA1* has been shown to depend on both *PIF*s and *PRR7* (Haydon et al., 2013; Shor et al., 2017). This hypothesis can be tested by assessing the clock behavior of *pifQ* and *pifQ*;*prr7* mutants under different concentrations of sucrose, as well as the binding of PIFs to *CCA1/LHY* promoters under different concentrations of sugar in both wild-type and *prr7* mutant backgrounds. This set of experiments will contribute to better understand the role and interaction of these key players within the system.

Simultaneously, PIFs could be the convergence point for additional endogenous (e.g., hormone pathways) and/or exogenous (e.g., temperature) signals. Thus, PIFs could integrate and convey multiple cues to the central clock, functioning as a calibration system able to fine-tune the biological oscillator by modulating features such as amplitude, pace, and resetting capability.

CRYPTOCHROMES AND CRYPTOCHROME SIGNALING

Cryptochromes are present in many different organisms, from bacteria to human, and despite their shared similarity with a group of light-activated DNA-repair enzymes known as photolyases, animal and plant cryptochromes have lost DNA-repair activity (Cashmore, 2003; Liu et al., 2016a). In *Arabidopsis*, the cryptochrome family encompasses three members, CRY1 (CRYPTOCHROME 1), CRY2, and CRY3, and while the physiological relevance of CRY3 remains to be clarified, CRY1 and CRY2 have been extensively characterized and are the focus of this section (Kleine et al., 2003; Chaves et al., 2011).

CRY1 and CRY2 play a major role as blue-light photoreceptors but they can sense a broader spectrum of wavelengths, including UV-A light (~390–480 nm) (Yu et al., 2010; Liu et al., 2016a). CRY2 is rapidly downregulated by blue light and functions primarily under low-fluence irradiances, whereas CRY1 is more stable and works at higher intensities of light (Lin et al., 1998). Both bind non-covalently to the chromophore flavin adenine dinucleotide, which is presumed to undergo a redox photocycle and trigger photochemical reactions that induce structural rearrangements in the protein (Zeugner et al., 2005; Ahmad, 2016). Photoexcited cryptochromes are subject to protein modifications and protein–protein interactions that allow them to mediate their biological function (Yu et al., 2010; Liu et al., 2016a; Yang et al., 2017).

CRY1 and CRY2 act as homodimers and through interaction with different partners are able to modulate gene expression, either by transcriptional or post-translational regulation. CRY2 associates with chromatin, likely through canonical transcription factors that positively or negatively regulate transcriptional activity (Pedmale et al., 2016). Thus, these photoreceptors are involved in a variety of blue-light-mediated physiological responses including de-etiolation, photomorphogenesis, flowering, and entrainment of the circadian clock (Yang et al., 2017).

Genetic studies have revealed that both CRY1 and CRY2 play important roles in the proper functioning of the endogenous oscillator and contribute to determining its features. Under continuous blue light, *cry1* mutants exhibit a lengthened free-running period compared with wild-type plants, but the magnitude of that difference is fluence rate dependent (Somers et al., 1998). *cry2* mutants have a more elusive phenotype and show slightly different results depending on the experimental setup (Somers et al., 1998; Devlin and Kay, 2000; Yanovsky et al., 2001). However, under continuous blue light, the double *cry1*;*cry2* mutant exhibits a longer period compared with wild-type or single-mutant plants, independent of fluence rate, suggesting a partial redundancy in the function of these two proteins on blue-light input to the clock (Devlin and Kay, 2000). Interestingly, both single and double *cry1*;*cry2* mutants also present differences on the period length under red light, and *cry1*;*cry2* mutants have also been found to participate in FR-light signaling, suggesting a crosstalk between the signaling pathways sensing these different wavelengths (see section below) (Devlin and Kay, 2000; Yanovsky et al., 2001). Additionally, the resetting ability of the clock in response to different light wavelengths was addressed in multiple genomic backgrounds, and the results also suggest that CRY1 and CRY2 are partially functionally redundant.

The mechanism linking the blue-light perception to the core of the biological oscillator is still not understood. Recent evidence showing that CRY2 binds several core-clock gene promoters provides a reasonable mode of action, especially considering that such association could be modulated by PIF transcription factors, which were already implicated on the fine-tuning of the oscillator (Pedmale et al., 2016). Besides PIFs, other hub molecules acting at the crossroad of multiple light wavelengths have been found to contribute to convey blue-light information (e.g., COP1; see below). Additional players regulating the core clock in response to this particular wavelength have been identified, although whether they are part of the cryptochrome signal transduction or other blue-light photoreceptors remain to be elucidated. For example, it

was shown that blue light is able to stabilize COR27 (COLD REGULATED GENE 27) and COR28 proteins, which bind to the promoters of *PRR5* and *TOC1* and repress their transcription (Li et al., 2016). Similarly, blue light induces *HY5* and *HYH* gene expression and protein accumulation. HY5, previously shown to be involved in PhyA signaling and known to regulate *ELF4* expression, was found to associate with many core-clock genes in a light-quality-dependent manner: binding to *CCA1*, *PRR9*, and *LUX*, among other promoters was increased under blue light and in the case of *PRR5*, *LUX*, and *BOA* (*BROTHER OF LUX ARRHYTHMO*), it correlated with changes in the expression level (Li et al., 2011a; Hajdu et al., 2018). Interestingly, the biological relevance of HY5/HYH on the functioning of the oscillator is revealed by the fact that the *hy5*;*hyh* double mutant has a shorter period than wild-type plants under free-running conditions either in darkness or white, blue, or red light, but the phenotype is more severe under blue light (Hajdu et al., 2018).

ZTL FAMILY OF PHOTORECEPTORS AND THEIR ROLE IN LIGHT INPUT TO THE CLOCK

Blue-light perception can also be achieved by the ZTL (ZEITLUPE) family of photoreceptors, comprising three members: ZTL, FKF1 (FLAVIN-BINDING, KELCH REPEAT, F-BOX 1), and LKP2 (LOV KELCH PROTEIN 2). The photosensory domain of these proteins is the LOV (light, oxygen, or voltage) domain, which binds non-covalently to the chromophore (flavin mononucleotide), and undergoes structural changes upon blue-light exposure that modulate the protein activity. Additionally, these photoreceptors exhibit two other functional domains: a Kelch repeat domain that mediates protein–protein interactions, and an F-box domain that allows these proteins to function as part of the E3 ubiquitin ligase Skp–Cullin–F-box (SCF) complex. This multimeric complex has the ability to trigger proteasomal-mediated protein degradation in a blue-light-dependent manner (Ito et al., 2012).

ZTL, LKP2, and FKF1 have been associated with control of the photoperiodic pathway of flowering and the circadian clock by modulating the accumulation of essential proteins in each network (Ito et al., 2012). As shown by single and high-order mutant analysis, these three proteins have partially overlapping functions on the control of the circadian oscillator, but ZTL appears to play a predominant role: *ztl* mutant plants exhibit either a long period or arrhythmic phenotype depending on the wavelength of irradiation, whereas *lkp2* and *fkf1* mutants do not present significant differences with wild-type plants (Jarillo et al., 2001; Baudry et al., 2010). Nevertheless, overexpressing lines of either *LKP2* or *ZTL* present an arrhythmic phenotype, supporting the idea that these *loci* play an overlapping but important role on the functioning of the circadian clock (Schultz et al., 2001; Somers et al., 2004).

ZTL interacts with GI in a blue-light-enhanced manner, and both proteins undergo a reciprocal co-stabilization depending on light (Kim et al., 2007, 2013). GI facilitates folding and maturation of ZTL, likely through the formation of a ternary complex with the chaperone HSP90 (HEAT SHOCK PROTEIN 90), which promotes ZTL stabilization (Kim et al., 2011; Cha et al., 2017). Additionally, GI can recruit either of two deubiquitylases (UBP12 [UBIQUITIN-SPECIFIC PROTEASE 12] or UBP13) to the ZTL–GI complex, contributing to ZTL protein stabilization and accumulation by the end of the light period (Lee et al., 2019). After dusk, the ZTL–GI complexes dissociate and ZTL triggers PRR5 and TOC1 proteasomal-mediated degradation by ubiquitination through the SCF complex (Mas et al., 2003; Kiba et al., 2007). Thus, modulation of PRR5 and TOC1 protein accumulation by ZTL in a light-dependent manner directly impinges on the pace of the biological clock. Similarly, ZTL has been shown to ubiquitylate CHE (CCA1 HIKING EXPEDITION), a TOC1-interacting protein that regulates *CCA1* expression, and control its stability in a light-dependent and ubiquitin proteasome system-dependent way (Pruneda-Paz et al., 2009; Lee et al., 2018). Furthermore, ZTL photocycle kinetics plays a fundamental role in determining the length of the circadian period. As shown by Pudasaini et al. (2017), plants carrying variants of ZTL with altered photocycle kinetics of the LOV domain present a different profile of PRR5 and TOC1 degradation, which correlates with the pace of the circadian clock. Considering that LOV domains have been proposed to distinguish fluctuations in light intensity, ZTL could be a critical player in the light-resetting mechanism of the plant clock, conveying information not only from the light to dark transition but also contributing to the parametric entrainment, which implies that the clock accelerates as the light fluence increases (Aschoff, 1979; Pudasaini and Zoltowski, 2013).

ULTRAVIOLET-B LIGHT SENSING AND SIGNALING

Visible light plays a central role in the physiological and developmental responses of *Arabidopsis*, with phytochromes and cryptochromes being the major photoreceptors of those wavelengths. Ultraviolet-B (UV-B) light (280–315 nm), although representing a minor portion of the sunlight spectrum, has a significant role in plant physiology. UV-B light can represent a stress factor, inducing DNA damage and impacting on development and growth, but at low intensities the same wavelengths can promote photomorphogenesis (Favory et al., 2009; Yin and Ulm, 2017).

UV-B light is sensed by UVR8 (UV Resistance Locus 8), which notably does not require the presence of a chromophore to convert the received photons into a biochemical signal (Rizzini et al., 2011). Instead, the photocycle of UVR8 is accomplished by a large number of aromatic residues within its structure, which induce the homodimerization of the protein and its retention in the cytoplasm. Upon UV-B light irradiation, the charges on the aromatic residues (tryptophan dominated) are redistributed, promoting the dissociation into active monomers. As a monomer, UVR8 is translocated to the nucleus and triggers regulatory changes in gene expression (Yin and Ulm, 2017; Podolec and Ulm, 2018).

UVR8 physically associates with different partners. It interacts with COP1 in a UV-B-dependent manner, promoting UVR8 nuclear accumulation (Favory et al., 2009; Rizzini et al., 2011). The

UVR8–COP1 interaction compromises the E3 ubiquitin ligase activity of COP1, which results in the reduction of ubiquitylation and degradation of the transcription factor HY5, and could therefore represent a mechanism to modify gene expression (Huang et al., 2013; Yin and Ulm, 2017; Podolec and Ulm, 2018; Liang et al., 2019). Interestingly, UVR8 and COP1 are both necessary for UV-B light entrainment of the circadian clock, but in an HY5/HYH-dispensable manner, suggesting an alternative signaling pathway to incorporate UV-B light information to the endogenous oscillator (Fehér et al., 2011). Consistently, UV-B light pulses induce transcription of several core-clock genes in an UVR8- and COP1-dependent manner. The level of transcriptional induction depends on the time of day at which the pulse is applied, suggesting that this response is gated by the circadian clock, giving rise to a loop of feedback regulation between UV-B light perception and the circadian-clock function (Fehér et al., 2011).

It has been shown that UV-B inhibits *PIF4* expression, thereby reducing PIF4 abundance. Despite the known roles of ELF3 and HY5 as transcriptional regulators of *PIF4*, these two factors were not found to be involved in the UV-B-mediated reduction of PIF4 levels (Nusinow et al., 2011; Delker et al., 2014; Hayes et al., 2017). Moreover, UV-B light triggers degradation of PIF4 and PIF5 and stabilizes the bHLH factor HFR1 (LONG HYPOCOTYL IN FAR RED 1), which can inhibit the ability of PIF4 and PIF5 to bind DNA, thereby repressing their transcriptional activity (Hornitschek et al., 2009; Hayes et al., 2014, 2017). Considering the recent evidence revealing that PIFs can repress *CCA1* expression, it is tempting to speculate that UV-B light information could be conveyed to the clockwork, at least partially, by modulating the abundance of PIFs (Nohales et al., 2019).

SIGNAL INTEGRATION AND CROSSTALK BETWEEN PATHWAYS

In nature, day/night cycles provide distinct types of information to plants about the surrounding environment. For example, the length of daylight, or photoperiod, has a decisive role on flowering time of many species. This is a key physiological response for plant survival, as well as of a great interest from an agronomic perspective. The current knowledge of both the role of photoperiod on synchronizing the circadian clock and the role of the biological oscillator on sensing this external variable to integrate it and modulate plant physiology have recently been reviewed (Oakenfull and Davis, 2017; Creux and Harmer, 2019; Webb et al., 2019).

Another important feature of the natural environment is the spectral composition of the incoming light, as it changes along the day and the year, and also depends on the presence of neighboring plants (Schäfer and Nagy, 2006). Therefore, is not surprising that plants have manifold strategies to integrate signals from different photoreceptors, each of which senses a particular wavelength. The crosstalk of light-signaling cascades occurs at almost every molecular level. Here, we describe just some of those convergence points, particularly those that are known or suspected to regulate the circadian clock.

Phytochromes are the canonical red/FR photoreceptors but they can also serve as blue-light sensors. In fact, several pieces of genetic data have shown that they participate in blue-light input to the circadian clock: resetting of *phyA* mutants under low-fluence blue light/dark cycles is impaired, they show a lengthened period under continuous blue light, and they exhibit a reduced phase shift compared with wild-type plants under pulses of this wavelength. Additionally, whereas the *cry1*;*cry2* mutant is less sensitive than wild-type plants to blue-light pulses, the triple mutant *phyA*;*cry1*;*cry2* is insensitive to that treatment, suggesting that these three loci are partially redundant components of blue-light input to the clock (Somers et al., 1998; Yanovsky et al., 2001). Conversely, *cry1*;*cry2* mutants exhibit a long period under continuous red light and CRY1 was proposed early on to act downstream of PhyA in white-light signaling to the clock (Devlin and Kay, 2000). This evidence shows that although different photoreceptors have a major role in sensing particular wavelengths, they are also able to sense others. Interestingly, PhyB and CRY2, as well as CRY1 and PhyA, are able to directly interact at the protein level, revealing a possible molecular target of convergence and regulation of different wavelengths (Figure 3) (Ahmad et al., 1998; Mas et al., 2000). Moreover, ZTL has also been shown to partner with both CRY1 and PhyB, increasing the complexity of this network (Jarillo et al., 2001).

CRY1 and CRY2 interact with PIF4 and PIF5, and CRY2 shares chromatin-binding regions with these two PIFs. Phenotypic analysis of different mutant combinations and biochemical assays suggest that CRYs regulate the ability of PIF4 and PIF5 to promote growth. Thus, PIFs become a molecular hub at which different photoreceptors converge to modulate at least some developmental responses (Pedmale et al., 2016). Research to address whether PIFs play a role in blue-light input to the clock has not yet been reported, but is of great interest as this could be a new link between CRYs and the clockwork, and could become a core integration point for diverse environmental signals.

The convergence of light-signaling cascades of distinct photoreceptor families on common components is becoming highly relevant, as these points of convergence could be key players in the integration of signals arising from the multiple wavelengths present in "white light" under which plants are growing. These pivotal components would integrate environmental light information and modulate downstream responses. One such element is COP1.

COP1 locus was first described by its role in repressing photomorphogenesis in darkness, and it was shown early on that such repressive activity is reversed by light (Deng et al., 1991). A lot of work was dedicated to understanding the molecular function of COP1 and the many partnerships established by this protein to modulate plant growth and development (Lau and Deng, 2012). COP1 is a RING E3 ubiquitin ligase that targets key regulators for proteasomal-mediated degradation. *In vivo*, COP1 functional activity depends on interaction with SPA accessory proteins. The SPA family comprises four members (SPA1 to SPA4), which exhibit partially overlapping functions and a differential pattern of expression. COP1 homodimerizes and associates with two SPA proteins. The assembled tetrameric complex,

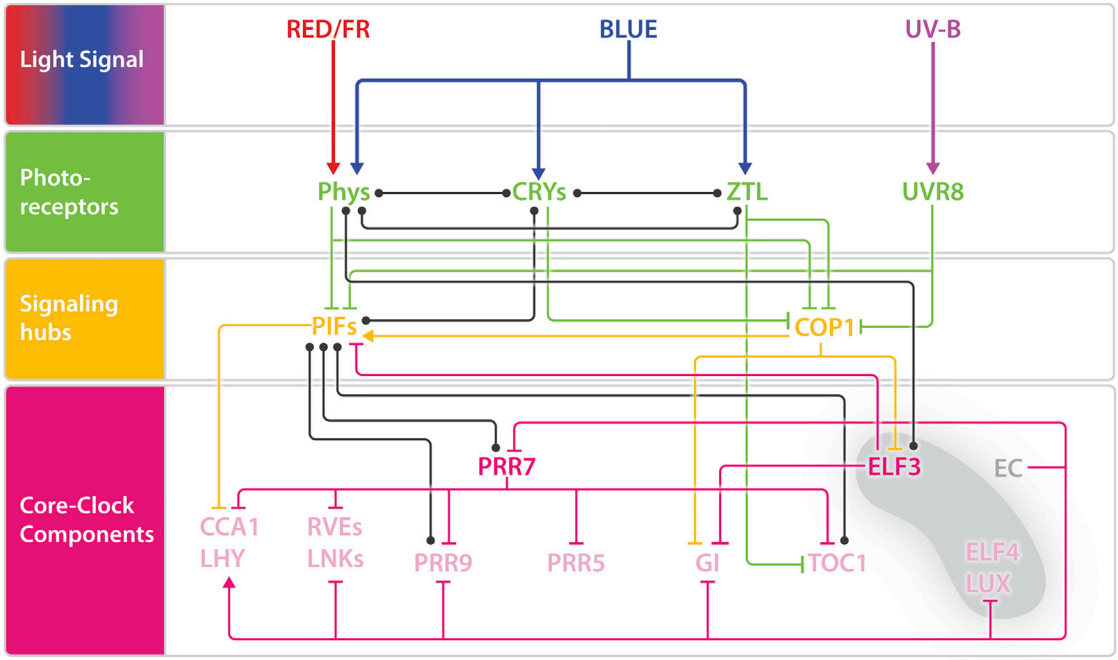

Figure 3. Conceptual Framework Highlighting the Crosstalk among Light-Signaling Pathways and Their Integration into the Circadian Clock Network.

Light signal is sensed by different groups of photoreceptors, which convey the environmental information to molecular signaling hubs. Core-clock components are targets of regulation of photoreceptors, signaling hubs, other clock proteins, and additional molecular players not shown in this figure. Multiple points of crosstalk among photoreceptors, signaling hubs, and other components of the network provide plasticity and accuracy to the system and allow the entrainment of the endogenous oscillator. Lines with blunt ends and arrows indicate repression and activation, respectively. The biological significance of the interaction between components linked by black, dot-ended lines has not been described. Green and orange lines represent photoreceptor- and signaling hubs-mediated regulation, respectively; pink lines indicate PRR7 or ELF3/EC regulatory steps (see text for more details). FR, far-red; UV-B, ultraviolet-B; EC, evening complex. Red, blue, and purple arrows, light signal; green-colored elements, photoreceptors; orange-colored elements, signaling hubs; pink-colored elements, core-clock components.

COP1–SPA, is supposed to be the substrate receptor of a bigger, multimeric complex known as the CUL4–DDB1 E3 ligase complex (Lau and Deng, 2012; Podolec and Ulm, 2018).

COP1 is known to be targeted by phytochromes, cryptochromes, UVR8, and FKF1. Upon light exposure, phytochromes and cryptochromes are known to repress COP1 activity by numerous modes of action that promote photomorphogenesis as well as other physiological responses (Podolec and Ulm, 2018). Conversely, the components of these two families of photoreceptors are negatively regulated by COP1–SPA, although not exactly through the same mechanism (Kim et al., 2017). Interestingly, mammalian cryptochromes, which are central components of the circadian clock, are not able to directly interact with the human COP1 ortholog but have retained their ability to negatively regulate its activity by interacting with a different protein of the human CUL4–RING ubiquitin complex (of which COP1 is a subunit). This suggests that the cryptochrome-mediated negative regulation of COP1 activity has been evolutionarily conserved (Rizzini et al., 2019).

As previously mentioned, UVR8 also signals through COP1. Activated UVR8 induces an increase in nuclear COP1 but the COP1–SPA complex gets separated from the CUL4–DDB1 E3 ligase complex, becoming inactive; therefore, some of its targets accumulate and trigger light responses (Podolec and Ulm, 2018).

Light-activated FKF1 can interact with and negatively regulate COP1 by inhibiting its homodimerization ability. This molecular regulation is associated with the modulation of flowering time (Lee et al., 2017). It would be interesting to assess whether FKF1 regulation of COP1 activity can also affect the ticking of the circadian clock.

ELF3 physically interacts with COP1 and bridges COP1 and GI association, which triggers degradation of GI. This is likely to occur upon transition from light to dark, when ELF3 and COP1 accumulate in the nucleus, and GI has been shown to undergo proteasome-mediated degradation. Thus, the GI accumulation pattern is modulated by ELF3 and COP1 in what could be considered a molecular link between photoreceptors that modulate COP1 activity and the core of the circadian clock, because modulating GI abundance will affect the biological oscillator (Yu et al., 2008). Moreover, upon its interaction with COP1, ELF3 becomes ubiquitylated and degraded by the proteasome, which could also affect the function of the EC and the clockwork itself (Yu et al., 2008). Consistently, it has been shown that *cop1* mutants exhibit circadian phenotypes (Millar et al., 1995; Yu et al., 2008).

The co-regulation among PIFs and the COP1–SPA complex provides an additional layer of complexity to the integration of light signals. PIF3 binds to the promoter and likely directly

regulates expression of SPA1; the COP1–SPA complex stabilizes PIFs in the dark (Martinez-Garcia et al., 2000; Pham et al., 2018a, 2018b). HY5 is a key transcription factor promoting photomorphogenesis, a known target of the COP1–SPA complex, and a hub where multiple signaling cascades converge. Repression of COP1 activity, mediated by light, allows an increase in HY5 abundance, which triggers photomorphogenesis. Interestingly, *hy5* mutant plants exhibit a short period length under continuous light of different wavelengths, but the phenotype is enhanced under blue light (Hajdu et al., 2018). Moreover, HY5 binds to the promoter of most of the core-clock genes under both red and blue wavelengths (preferably under the latter), which correlates with an induction of HY5 upon exposure to blue light at both transcriptional and post-transcriptional levels. As a molecular hub, HY5 interacts with a myriad of transcription factors to coordinately regulate gene expression. It has been proposed that the light-quality-dependent composition of those complexes (i.e., having more or less HY5 availability) could modulate the functioning of the circadian clock as a response to the wavelength composition of the white light under which plants are growing (Hajdu et al., 2018). Additionally, HY5 directly interacts with CCA1, and it has been suggested that CCA1 could contribute to the recruitment of HY5 to certain promoters during the early morning when CCA1 peaks and light inactivation of COP1 allows the accumulation of HY5 (Andronis et al., 2008).

DET1 (DE-ETIOLATED 1) is part of the CDD complex (COP10–DET1–CCB1) which has a key role on repressing photomorphogenesis. Its action has not been associated with a particular wavelength but with general light signaling. Specifically, DET1 directly interacts with CCA1 and LHY, which is believed to allow its recruitment to the promoter of *TOC1*, such that DET1 represses transcription of *TOC1*. *det1* mutant plants exhibit a short period phenotype, suggesting that this protein is essential for determining the pace of the oscillator (Millar et al., 1995; Lau et al., 2011). Additionally, DET1 positively regulates PIF abundance in the dark, although whether this constitutes another input pathway to the oscillator has not been addressed (Dong et al., 2014).

The *CSU4* (*COP1 SUPPRESSOR 4*) locus was identified by its genetic role as a suppressor of the *cop1* mutant phenotype. Although the gene encodes a protein with a domain of unknown function, the molecular characterization showed that CSU4 directly binds CCA1 and represses its transcriptional repression activity. Likely as a consequence, *csu4* mutants exhibit increased levels of *CCA1* and *PIF4* (Zhao et al., 2018).

Finally, the family of PPKs (PHOTOREGULATORY PROTEIN KINASES 1 to 4, previously known as MLKs [MUT9-LIKE KINASES]) could also represent a convergence point for the input of multiple light wavelengths to the clock. *ppk2*, *ppk3*, and *ppk4* single mutants (*mlk1*, *mlk2*, *mlk3*, respectively) exhibit a long period phenotype, which is rescued by a *ppk1* (*mlk4*) mutant allele through an unknown mechanism. Interestingly, PPKs associate *in vivo* with ELF3 (Huang et al., 2016a). PPKs also interact with PIF3 and PhyB in a light-induced manner and are required for light-mediated phosphorylation and degradation of PIF3 (Ni et al., 2017). PPKs additionally associate with and phosphorylate photoexcited CRY2, which is proposed to activate and destabilize the photoreceptor (Liu et al., 2017). Thus, this recently studied family of kinases could play a central role to integrate and transduce the signal of multiple wavelengths perceived by phytochromes and cryptochromes by affecting PIF3 accumulation and/or through their interaction with ELF3, ultimately affecting circadian-clock rhythmicity.

ELF3 AND PRR7 AS KEY INTEGRATORS

Different pieces of evidence led to the broadly accepted view that the *Arabidopsis* clock is set on time mainly by the transition from dark to light (Millar and Kay, 1996; McWatters et al., 2000; Seaton et al., 2018). Data from other organisms suggest that either the night length or both dawn and dusk information synchronize their endogenous oscillators (Hayama et al., 2018; Ramos-Sánchez et al., 2019). Nevertheless, considering that light (through PIF inactivation) activates the expression of *CCA1*, that metabolic status (defined by sugar levels) modulates *PRR7*, and that dusk (through ZTL) induces TOC1 degradation, the more recently proposed hypothesis of a dynamic and continuous entrainment fits best with the observed behavior of the circadian system in *Arabidopsis* (Mas et al., 2003; Haydon et al., 2013; Frank et al., 2018; Seaton et al., 2018; Nohales et al., 2019; Webb et al., 2019).

The large number of elements constituting the oscillator and their intricate cross-regulation makes the prediction of the circadian network behavior difficult, even through mathematical modeling (Pokhilko et al., 2012; Fogelmark and Troein, 2014). However, a new approach proposes to conceive the clock as a binary system with two states: morning and evening, characterized by CCA1/LHY and TOC1/PRR5 activity, respectively. In this model there are two rapid switchers that allow the system to move forward and transition from one state to the other: PRR9/PRR7 are the elements pushing the system from the morning to the evening state; the EC does the opposite (Joanito et al., 2018). Interestingly, PRR7 and ELF3 (one of the three components of the EC) both have a set of particular features that support the hypothesis of these two genes as particularly important within the circadian network and, perhaps, reliable components to determine the switch of the system from the morning to the night state and vice versa (Figure 3).

PRR7 plays a partially redundant role with *PRR9*. However, *prr7* single mutants exhibit a longer period than wild-type plants and are insensitive to different red fluence rates, whereas the wild-type genotype exhibits a shortening of the period as the irradiated light increases (as expected by Aschoff's rule) (Aschoff, 1979; Farré et al., 2005). This suggests that PRR7 is involved in red-light input to the clock. Notably, analogous results are obtained for *prr9* mutants under blue light, which raises the question of whether these two genes function complementarily for this response under these two wavelengths (Farré et al., 2005). *PRR7* is necessary to gate the clock resetting driven by sugar, as *prr7* mutants induce the same phase shift to a pulse of exogenous sucrose independently of the time of the day at which the treatment is applied (Haydon et al., 2013). Additionally, the *prr7* mutant period length is not adjusted to different concentrations of nicotinamide, a metabolite known to slow down the pace of the clock (Mombaerts et al., 2019).

PRR7 is also necessary for the proper response of the circadian clock to different temperature treatments (Salomé and McClung, 2005). In summary, the evidence suggests that *PRR7* is essential for red light, sugar, other metabolites (such as nicotinamide), and temperature input to the clock.

ELF3 is required to sustain rhythms under continuous light but not under continuous darkness. Overexpressing *ELF3* lines show an increased period length in both constant blue and red light, suggesting that *ELF3* is associated with input of information from both wavelengths to the clockwork (Covington et al., 2001). *ELF3* is also necessary to properly entrain the clock to thermocycles (Thines and Harmon, 2010). Besides its role as a transcriptional regulator, ELF3 is described to act as a hub protein and has been found to associate *in vivo* with the five members of the phytochrome family, as well as with many other elements of the red and FR light-signaling pathways (Huang et al., 2016a). ELF3 has been shown to interact with both forms of PhyB (Pr and Pfr), suggesting that these two proteins could partner either in the nucleus or in the cytoplasm (Liu et al., 2001; Yeom et al., 2014). Finally, under dark conditions, ELF3 bridges COP1 and GI, which triggers COP1-mediated degradation of GI and could indirectly affect ZTL accumulation and ZTL-mediated degradation of TOC1. These steps likely contribute to setting the time by conveying the "dark signal" to the core clock (Nohales and Kay, 2016). Additionally, the direct association of COP1 with ELF3 links the latter to virtually every light-signaling cascade, as COP1 is a common player to all.

Both PRR7 and ELF3 have been shown to partner with PIFs, although the biological relevance of such interactions has not been clarified for PRR7 and has been associated with the control of output responses but not to clock input for ELF3 (Nieto et al., 2015; Martín et al., 2018). PIFs and PRR7 are also targeted by other molecular pathways, conveying to the clock other input signals. This evidence led us to propose that PIFs and COP1 (described above as fundamental components of light-signaling pathways) act downstream of the photoreceptors and make up a first layer of signal integration. PRR7 and ELF3 could thus represent a second layer of signal integration and, at the same time, core-clock components (Figure 3).

FEEDBACK SIGNALING OF THE CLOCK INTO LIGHT PERCEPTION

Light perception and its signaling cascades are modulated by the endogenous clock at different levels. This is not an exclusive feature of light-sensing pathways, as it is known that the biological oscillator creates signaling feedback loops with several of its entraining cues as well as in its downstream responses, which both contribute to its fine-tuning (Sanchez and Kay, 2016).

The first clue suggesting that the endogenous clock has the ability to modulate light responses originated from gating experiments in which the induction (or phase shift) of a clock target gene was assessed after plants were treated with light pulses at different times of the day (Millar and Kay, 1996). With the molecular identification of clock components, the loci associated with this response, as well as the wavelength specificity, were also addressed (McWatters et al., 2000). The mechanism underlying this control is not yet completely understood, but some pieces of the puzzle have been fitted in place (Oakenfull and Davis, 2017).

Members of the phytochrome family exhibit a circadian profile of expression, peaking at different times of the day (Harmer et al., 2000; Schaffer et al., 2001; Tóth et al., 2001). It has been hypothesized that such patterns of mRNA accumulation could imply a physiological role, as *PhyB* (which encodes a photostable photoreceptor known to mediate responses under high-fluence-rate wavelengths) reaches its maximum level of expression around mid-morning, which correlates with high light intensities in natural environments. On the other hand, PhyA (a photolabile molecule associated with low fluence rate and FR-light responses) shows a peak of mRNA expression at the end of the day, which correlates with low light intensities and enrichment of FR wavelengths under natural conditions (Tóth et al., 2001). Nevertheless, at the protein level, PhyA exhibits a peak at the end of the night under light–dark conditions, whereas PhyB and PhyE accumulations do not present significant changes in amplitude and PhyC displays only a very weak oscillation under the same growth conditions (Sharrock and Clack, 2002). Consistently, PhyB protein level in tobacco plants has been shown to be fairly stable throughout the day under light–dark conditions (Bognár et al., 1999). Moreover, PhyA, PhyB, PhyC, and PhyE do not show significant changes in their protein levels under circadian conditions (continuous light) (Sharrock and Clack, 2002), so it remains to be elucidated whether the circadian profile of mRNA expression of phytochromes is biologically relevant. It has been shown, however, that subcellular localization of phytochromes is regulated by different wavelengths and affects signaling ability (Li et al., 2011b). Additionally, it is conceivable that the circadian clock modulates the signaling ability of PhyB through PCH1 because PCH1 is required for formation of photobodies (which is in turn necessary for PhyB signaling and regulation of numerous physiological and developmental processes; see "Phytochrome Signaling"), and *PCH1* mRNA expression and protein levels exhibit a circadian profile of oscillation, peaking in the evening. Together, these data suggest that the biological clock contributes to the timing of light responses by modulating the PhyB-signaling pathway (Huang et al., 2016b).

PIFs play an important role in the feedback signaling of the clock to light input, as they can induce PhyB degradation and regulate *PhyA* expression and PhyA activity (Leivar et al., 2008, 2012; Seaton et al., 2018). As explained above, PIFs and core-clock elements are tightly linked: *PIFs* are direct transcriptional targets of core-clock components and their encoded proteins interact with multiple clockwork elements to coordinate downstream responses. It is possible that PIFs–clock interactions may also modulate the regulatory role of PIFs on phytochromes, although to date no evidence has been found to support this hypothesis.

The cryptochrome profile of expression is also under the control of the circadian clock and resembles that of the phytochrome family: *CRY1* peaks in the late morning whereas *CRY2* (the protein of which is rapidly downregulated by light) reaches its maximum at the end of the day, consistent with the light-dependent stability and biological function of each photoreceptor (Lin et al., 1998; Harmer et al., 2000; Tóth et al., 2001). As already

discussed, cryptochromes act in concert with PIF4 and PIF5 to modulate downstream gene expression (Pedmale et al., 2016). Hence, if core-clock components impinge on PIF activity, this could be an additional way of the endogenous timekeeper modulating blue-light responses.

Despite the *ZTL* expression level not exhibiting a circadian profile, ZTL abundance does show a rhythmic pattern of accumulation under light/dark cycles reaching its peak at the end of the day, which correlates with blue-light enrichment in natural conditions and could contribute to its physiological role associated with regulation of TOC1 and PRR5 and CHE stability (Kim et al., 2003b; Nohales and Kay, 2016). UV-B light signaling is also gated by the circadian clock, an example of the feedback regulation of the clockwork (Fehér et al., 2011; Takeuchi et al., 2014). However, the level of UVR8 monomer is relatively constant under diurnal conditions, suggesting that the clock and UV-B light-signaling networks interact at a different level (Findlay and Jenkins, 2016).

ENTRAINMENT OF THE CLOCK: A WIDER PERSPECTIVE

Crosstalk between wavelengths and feedback from the core clock to the light perception shape a convoluted network of interactions (Figure 3). However, this system is more complex still, as it includes many additional synchronizing cues. First, light allows plants to produce sugar through photosynthesis. It has been shown that the rhythmic metabolic state driven by sugars can entrain the circadian clock, representing an indirect mechanism for light input to the clock (Haydon et al., 2013; Sanchez and Kay, 2016; Frank et al., 2018). Second, hormone levels are circadian modulated and can in turn regulate different parameters of the pacemaker (Thain et al., 2004; Nováková et al., 2005; Hanano et al., 2006; Covington et al., 2008; Legnaioli et al., 2009; Lee et al., 2016; Sanchez and Kay, 2016; Zhang et al., 2019b). Because the interaction between light- and hormone-signaling pathways has been established, it introduces an additional indirect path for light to modulate the biological rhythms (Leivar and Monte, 2014; Paik and Huq, 2019). Third, temperature, together with light, is a fundamental variable for biological clock entrainment (McClung, 2019). Temperature is known to input to the clock at different levels including regulating *LUX* at the transcriptional level, modulating alternative splicing of several core-clock genes and through *PRR7* and *PRR9* by an unknown mechanism (Salomé and McClung, 2005; Chow et al., 2014; Mateos et al., 2018). Furthermore, PhyB has the ability to act as a thermosensor, so temperature-mediated regulation of PhyB could contribute to setting the pace of the core clock (Jung et al., 2016; Legris et al., 2016). Lastly, although not described here, it is important to note that other endogenous and exogenous variables, including biotic and abiotic stress and nutrient homeostasis, can fine-tune the functioning of the biological oscillator (Haydon et al., 2015; Seo and Mas, 2015).

CONCLUDING REMARKS

The circadian system regulates virtually every aspect of plant growth and development; therefore, understanding how it is synchronized is of great importance to our ability to predict how changes in environmental stimuli will affect endogenous rhythms and, consequently, plant physiology. It has been clearly established that light is one of the key entrainment factors of the internal oscillator, and many efforts have been focused over the last 20 years on describing the mechanism underlying that process. However, despite significant advances made and many pieces uncovered, a comprehensive view and understanding of its functioning, is still lacking. Likely, the high degree of crosstalk and feedback between multiple endogenous and external cues that signal back and forth to the core clock make difficult the unraveling of the entrainment system (Figure 3). System-level approaches, together with mathematical modeling and genome-scale analysis, have contributed to the elucidation of this complex network, as they have identified missing pieces and/or connections, which would have been difficult to find by traditional methods (Gould et al., 2013; Joanito et al., 2018; Seaton et al., 2018).

Protein dynamics, including protein–protein and protein–chromatin interactions, as well as the post-translational modifications required for such associations, represent essential regulatory steps that have been poorly explored. In fact, specific examples of the importance of such dynamics have been identified. The COP1–ELF3 interaction has been suggested to be dark dependent (Yu et al., 2008). PhyB appears to associate with different clock components depending on its Pr or Pfr state (Yeom et al., 2014). Remarkably, *in vivo* experiments have shown the five phytochromes interacting with ELF3, despite the fact that these photoreceptors are thought to be mainly active during the daytime, whereas ELF3 tends toward the night (Huang et al., 2016a). Additionally, many interactions are known to be dependent on subcellular localization and post-translational modifications (Nohales and Kay, 2016). Many researchers have deployed strategies to assess context-free interactions (yeast two-hybrid or *in vitro* assays; Table 2), major contributions that provide the opportunity to overcome functional redundancy and evaluate direct interaction but also have limitations. Hence, to answer the important questions of where (within the cell or the plant) and when different proteins associate, as well as which post-translational modifications are required for those interactions to occur, *in vivo* experiments should be carried out. The additional information gained from such approaches will likely contribute to comprehension of the biological relevance of those partnerships, and ultimately enrich our understanding of the mechanisms underlying clock entrainment.

FUNDING

S.E.S. is partially supported by a postdoctoral fellowship from the Pew Latin American Fellows Program. This work is supported by the National Institute of General Medical Sciences of the National Institutes of Health under award nos. R01GM067837 and R01GM56006. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

ACKNOWLEDGMENTS

The authors apologize for not citing all relevant publications by colleagues because of space limitations. No conflict of interest declared.

Received: September 3, 2019
Revised: February 10, 2020
Accepted: February 12, 2020
Published: February 13, 2020

REFERENCES

Adams, S., Manfield, I., Stockley, P., and Carré, I.A. (2015). Revised morning loops of the *Arabidopsis* circadian clock based on analyses of direct regulatory interactions. PLoS One **10**:e0143943.

Ahmad, M. (2016). Photocycle and signaling mechanisms of plant cryptochromes. Curr. Opin. Plant Biol. **33**:108–115.

Ahmad, M., Jarillo, J.A., Smirnova, O., and Cashmore, A.A. (1998). The CRY1 blue light photoreceptor of *Arabidopsis* interacts with phytochrome A in vitro. Mol. Cell **1**:939–948.

Alabadí, D., Oyama, T., Yanovsky, M.J., Harmon, F.G., Mas, P., and Kay, S.A. (2001). Reciprocal regulation between TOC1 and LHY/CCA1 within the *Arabidopsis* circadian clock. Science **293**:880–883.

Allen, T., Koustenis, A., Theodorou, G., Somers, D.E., Kay, S.A., Whitelam, G.C., and Devlin, P.F. (2006). *Arabidopsis* FHY3 specifically gates phytochrome signaling to the circadian clock. Plant Cell **18**:2506–2516.

Anderson, S.L., Somers, D.E., Millar, A.J., Hanson, K., Chory, J., and Kay, S.A. (1997). Attenuation of phytochrome A and B signaling pathways by the *Arabidopsis* circadian clock. Plant Cell **9**:1721–1743.

Andronis, C., Barak, S., Knowles, S.M., Sugano, S., and Tobin, E.M. (2008). The clock protein CCA1 and the bZIP transcription factor HY5 physically interact to regulate gene expression in *Arabidopsis*. Mol. Plant **1**:58–67.

Aschoff, J. (1979). Circadian rhythms: influences of internal and external factors on the period measured in constant conditions1. Z. Tierpsychol. **49**:225–249.

Baudry, A., Ito, S., Song, Y.H., Strait, A.A., Kiba, T., Lu, S., Henriques, R., Pruneda-Paz, J.L., Chua, N.H., Tobin, E.M., et al. (2010). F-box proteins FKF1 and LKP2 act in concert with ZEITLUPE to control *Arabidopsis* clock progression. Plant Cell **22**:606–622.

Bognár, L.K., Hall, A., Ádám, É., Thain, S.C., Nagy, F., and Millar, A.J. (1999). The circadian clock controls the expression pattern of the circadian input photoreceptor, phytochrome B. Proc. Natl. Acad. Sci. U S A **96**:14652–14657.

Briggs, W.R. (2006). Blue/UV-A receptors: historical overview. In Photomorphogenesis in Plants and Bacteria, E. Schafer and F. Nagy, eds. (Dordrecht: Springer Netherlands), pp. 171–197.

Casal, J.J. (2013). Photoreceptor signaling networks in plant responses to shade. Annu. Rev. Plant Biol. **64**:403–427.

Casal, J.J., and Qüesta, J.I. (2018). Light and temperature cues: multitasking receptors and transcriptional integrators. New Phytol. **217**:1029–1034.

Cashmore, A.R. (2003). Cryptochromes: enabling plants and animals to determine circadian time. Cell **114**:537–543.

Castillon, A., Shen, H., and Huq, E. (2007). Phytochrome Interacting Factors: central players in phytochrome-mediated light signaling networks. Trends Plant Sci. **12**:514–521.

Cha, J.Y., Kim, J., Kim, T.S., Zeng, Q., Wang, L., Lee, S.Y., Kim, W.Y., and Somers, D.E. (2017). GIGANTEA is a co-chaperone which facilitates maturation of ZEITLUPE in the *Arabidopsis* circadian clock. Nat. Commun. **8**:3.

Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L.-O., Horst, G.T.J.v.d., Batschauer, A., and Ahmad, M. (2011). The cryptochromes: blue light photoreceptors in plants and animals. Annu. Rev. Plant Biol. **62**:335–364.

Chen, F., Li, B., Li, G., Charron, J.B., Dai, M., Shi, X., and Deng, X.W. (2014). *Arabidopsis* phytochrome A directly targets numerous promoters for individualized modulation of genes in a wide range of pathways. Plant Cell **26**:1949–1966.

Chow, B.Y., Sanchez, S.E., Breton, G., Pruneda-Paz, J.L., Krogan, N.T., and Kay, S.A. (2014). Transcriptional regulation of LUX by CBF1 mediates cold input to the circadian clock in *Arabidopsis*. Curr. Biol. **24**:1518–1524.

Clack, T., Shokry, A., Moffet, M., Liu, P., Faul, M., and Sharrock, R.A. (2009). Obligate heterodimerization of *Arabidopsis* phytochromes C and E and interaction with the PIF3 basic helix-loop-helix transcription factor. Plant Cell **21**:786–799.

Covington, M.F., Panda, S., Liu, X.L., Strayer, C.A., Wagner, D.R., and Kay, S.A. (2001). ELF3 modulates resetting of the circadian clock in *Arabidopsis*. Plant Cell **13**:1305–1315.

Covington, M., Maloof, J., Straume, M., Kay, S., and Harmer, S. (2008). Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. Genome Biol. **9**:R130.

Creux, N., and Harmer, S. (2019). Circadian rhythms in plants. Cold Spring Harb. Perspect. Biol. https://doi.org/10.1101/cshperspect.a034611.

Delker, C., Sonntag, L., James, G.V., Janitza, P., Ibañez, C., Ziermann, H., Peterson, T., Denk, K., Mull, S., Ziegler, J., et al. (2014). The DET1-COP1-HY5 pathway constitutes a multipurpose signaling module regulating plant photomorphogenesis and thermomorphogenesis. Cell Rep. **9**:1983–1989.

Deng, X.W., Caspar, T., and Quail, P.H. (1991). *cop*1: a regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*. Genes Dev. **5**:1172–1182.

Devlin, P.F., and Kay, S.A. (2000). Cryptochromes are required for phytochrome signaling to the circadian clock but not for rhythmicity. Plant Cell **12**:2499–2509.

Dodd, A.N., Salathia, N., Hall, A., Kevei, E., Toth, R., Nagy, F., Hibberd, J.M., Millar, A.J., and Webb, A.A.R. (2005). Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science **309**:630–633.

Dong, J., Tang, D., Gao, Z., Yu, R., Li, K., He, H., Terzaghi, W., Deng, X.W., and Chen, H. (2014). *Arabidopsis* DE-ETIOLATED1 represses photomorphogenesis by positively regulating phytochrome-interacting factors in the dark. Plant Cell **26**:3630–3645.

Doyle, M.R., Davis, S.J., Bastow, R.M., McWatters, H.G., Kozma-Bognar, L., Nagy, F., Millar, A.J., and Amasino, R.M. (2002). The ELF4 gene controls circadian rhythms and flowering time in *Arabidopsis thaliana*. Nature **419**:74–77.

Fankhauser, C., and Staiger, D. (2002). Photoreceptors in *Arabidopsis thaliana*: light perception, signal transduction and entrainment of the endogenous clock. Planta **216**:1–16.

Farinas, B., and Mas, P. (2011). Functional implication of the MYB transcription factor RVE8/LCL5 in the circadian control of histone acetylation. Plant J. **66**:318–329.

Farré, E.M., Harmer, S.L., Harmon, F.G., Yanovsky, M.J., and Kay, S.A. (2005). Overlapping and distinct roles of PRR7 and PRR9 in the *Arabidopsis* circadian clock. Curr. Biol. **15**:47–54.

Favory, J.J., Stec, A., Gruber, H., Rizzini, L., Oravecz, A., Funk, M., Albert, A., Cloix, C., Jenkins, G.I., Oakeley, E.J., et al. (2009). Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in *Arabidopsis*. EMBO J. **28**:591–601.

Fehér, B., Kozma-Bognár, L., Kevei, É., Hajdu, A., Binkert, M., Davis, S.J., Schäfer, E., Ulm, R., and Nagy, F. (2011). Functional interaction of the circadian clock and UV RESISTANCE LOCUS 8-controlled UV-B signaling pathways in *Arabidopsis thaliana*. Plant J. **67**:37–48.

Findlay, K.M., and Jenkins, G.I. (2016). Regulation of UVR8 photoreceptor dimer/monomer photo-equilibrium in *Arabidopsis* plants grown under photoperiodic conditions. Plant Cell Environ. **39**:1706–1714.

Fogelmark, K., and Troein, C. (2014). Rethinking transcriptional activation in the *Arabidopsis* circadian clock. PLoS Comput. Biol. **10**:e1003705.

Frank, A., Matiolli, C.C., Viana, A.J.C., Hearn, T.J., Kusakina, J., Belbin, F.E., Wells Newman, D., Yochikawa, A., Cano-Ramirez, D.L., Chembath, A., et al. (2018). Circadian entrainment in arabidopsis by the sugar-responsive transcription factor bZIP63. Curr. Biol. **28**:2597–2606.

Franklin, K.A., and Quail, P.H. (2010). Phytochrome functions in *Arabidopsis* development. J. Exp. Bot. **61**:11–24.

Fujimori, T., Yamashino, T., Kato, T., and Mizuno, T. (2004). Circadian-controlled basic/helix-loop-helix factor, PIL6, implicated in light-signal transduction in *Arabidopsis thaliana*. Plant Cell Physiol. **45**:1078–1086.

Fujiwara, S., Wang, L., Han, L., Suh, S.S., Salomé, P.A., McClung, C.R., and Somers, D.E. (2008). Post-translational regulation of the *Arabidopsis* circadian clock through selective proteolysis and phosphorylation of pseudo-response regulator proteins. J. Biol. Chem. **283**:23073–23083.

Gangappa, S.N., and Botto, J.F. (2016). The multifaceted roles of HY5 in plant growth and development. Mol. Plant **9**:1353–1365.

Gil, K.E., and Park, C.M. (2019). Thermal adaptation and plasticity of the plant circadian clock. New Phytol. **221**:1215–1229.

Gould, P.D., Ugarte, N., Domijan, M., Costa, M., Foreman, J., MacGregor, D., Rose, K., Griffiths, J., Millar, A.J., Finkenstädt, B., et al. (2013). Network balance via CRY signalling controls the *Arabidopsis* circadian clock over ambient temperatures. Mol. Syst. Biol. **9**:650.

Greenham, K., and McClung, C.R. (2015). Integrating circadian dynamics with physiological processes in plants. Nat. Rev. Genet. **16**:598–610.

Hajdu, A., Ádám, É., Sheerin, D.J., Dobos, O., Bernula, P., Hiltbrunner, A., Kozma-Bognár, L., and Nagy, F. (2015). High-level expression and phosphorylation of phytochrome B modulates flowering time in *Arabidopsis*. Plant J. **83**:794–805.

Hajdu, A., Dobos, O., Domijan, M., Bálint, B., Nagy, I., Nagy, F., and Kozma-Bognár, L. (2018). ELONGATED HYPOCOTYL 5 mediates blue light signalling to the *Arabidopsis* circadian clock. Plant J. **96**:1242–1254.

Hall, A., Kozma-Bognár, L., Bastow, R.M., Nagy, F., and Millar, A.J. (2002). Distinct regulation of CAB and PHYB gene expression by similar circadian clocks. Plant J. **32**:529–537.

Hanano, S., Domagalska, M.A., Nagy, F., and Davis, S.J. (2006). Multiple phytohormones influence distinct parameters of the plant circadian clock. Genes Cells **11**:1381–1392.

Harmer, S.L., Hogenesch, J.B., Straume, M., Chang, H.-S., Han, B., Zhu, T., Wang, X., Kreps, J.A., and Kay, S.A. (2000). Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. Science **290**:2110–2113.

Hayama, R., Mizoguchi, T., and Coupland, G. (2018). Differential effects of light-to-dark transitions on phase setting in circadian expression among clock-controlled genes in *Pharbitis nil*. Plant Signal. Behav. **13**:e1473686.

Haydon, M.J., Mielczarek, O., Robertson, F.C., Hubbard, K.E., and Webb, A.A.R. (2013). Photosynthetic entrainment of the *Arabidopsis thaliana* circadian clock. Nature **502**:689–692.

Haydon, M.J., Román, Á., and Arshad, W. (2015). Nutrient homeostasis within the plant circadian network. Front. Plant Sci. **6**:299.

Hayes, S., Velanis, C.N., Jenkins, G.I., and Franklin, K.A. (2014). UV-B detected by the UVR8 photoreceptor antagonizes auxin signaling and plant shade avoidance. Proc. Natl. Acad. Sci. U S A **111**:11894–11899.

Hayes, S., Sharma, A., Fraser, D.P., Trevisan, M., Cragg-Barber, C.K., Tavridou, E., Fankhauser, C., Jenkins, G.I., and Franklin, K.A. (2017). UV-B perceived by the UVR8 photoreceptor inhibits plant thermomorphogenesis. Curr. Biol. **27**:120–127.

Helfer, A., Nusinow, D.A., Chow, B.Y., Gehrke, A.R., Bulyk, M.L., and Kay, S.A. (2011). LUX ARRHYTHMO encodes a nighttime repressor of circadian gene expression in the *Arabidopsis* core clock. Curr. Biol. **21**:126–133.

Hicks, K.A., Millar, A.J., Carre, I.A., Somers, D.E., Straume, M., Meeks-Wagner, D.R., and Kay, S.A. (1996). Conditional circadian dysfunction of the *Arabidopsis early-flowering 3* mutant. Science **274**:790–792.

Hornitschek, P., Lorrain, S., Zoete, V., Michielin, O., and Fankhauser, C. (2009). Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. EMBO J. **28**:3893–3902.

Hornitschek, P., Kohnen, M.V., Lorrain, S., Rougemont, J., Ljung, K., López-Vidriero, I., Franco-Zorrilla, J.M., Solano, R., Trevisan, M., Pradervand, S., et al. (2012). Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. Plant J. **71**:699–711.

Hsu, P.Y., Devisetty, U.K., and Harmer, S.L. (2013). Accurate timekeeping is controlled by a cycling activator in *Arabidopsis*. eLife **2**:e00473.

Hu, W., Franklin, K.A., Sharrock, R.A., Jones, M.A., Harmer, S.L., and Lagarias, J.C. (2013). Unanticipated regulatory roles for *Arabidopsis* phytochromes revealed by null mutant analysis. Proc. Natl. Acad. Sci. U S A **110**:1542–1547.

Huang, H., and Nusinow, D.A. (2016). Into the evening: complex interactions in the *Arabidopsis* circadian clock. Trends Genet. **32**:674–686.

Huang, W., Pérez-García, P., Pokhilko, A., Millar, A.J., Antoshechkin, I., Riechmann, J.L., and Mas, P. (2012). Mapping the core of the *Arabidopsis* circadian clock defines the network structure of the oscillator. Science **336**:75–79.

Huang, X., Ouyang, X., Yang, P., Lau, O.S., Chen, L., Wei, N., and Deng, X.W. (2013). Conversion from CUL4-based COP1-SPA E3 apparatus to UVR8-COP1-SPA complexes underlies a distinct biochemical function of COP1 under UV-B. Proc. Natl. Acad. Sci. U S A **110**:16669–16674.

Huang, H., Alvarez, S., Bindbeutel, R.K., Shen, Z., Naldrett, M.J., Evans, B.S., Briggs, S.P., Hicks, L.M., Kay, S.A., and Nusinow, D.A. (2016a). Identification of evening complex associated proteins in *Arabidopsis* by affinity purification and mass spectrometry. Mol. Cell Proteomics **15**:201–217.

Huang, H., Yoo, C.Y., Bindbeutel, R., Goldsworthy, J., Tielking, A., Alvarez, S., Naldrett, M.J., Evans, B.S., Chen, M., and Nusinow, D.A. (2016b). PCH1 integrates circadian and light-signaling pathways to control photoperiod-responsive growth in *Arabidopsis*. eLife **5**:e13292.

Huang, X., Zhang, Q., Jiang, Y., Yang, C., Wang, Q., and Li, L. (2018). Shade-induced nuclear localization of PIF7 is regulated by phosphorylation and 14-3-3 proteins in *Arabidopsis*. Elife **7**:e31636.

Huang, H., McLoughlin, K.E., Sorkin, M.L., Burgie, E.S., Bindbeutel, R.K., Vierstra, R.D., and Nusinow, D.A. (2019). PCH1 regulates light, temperature, and circadian signaling as a structural component of phytochrome B-photobodies in *Arabidopsis*. Proc. Natl. Acad. Sci. U S A **116**:8603–8608.

Ito, S., Song, Y.H., and Imaizumi, T. (2012). LOV domain-containing F-box proteins: light-dependent protein degradation modules in *Arabidopsis*. Mol. Plant **5**:573–582.

Jarillo, J.A., Capel, J., Tang, R.-H., Yang, H.-Q., Alonso, J.M., Ecker, J.R., and Cashmore, A.R. (2001). An *Arabidopsis* circadian clock component interacts with both CRY1 and phyB. Nature **410**:487–490.

Joanito, I., Chu, J.-W., Wu, S.-H., and Hsu, C.-P. (2018). An incoherent feed-forward loop switches the *Arabidopsis* clock rapidly between two hysteretic states. Sci. Rep. **8**:13944.

Jones, M.A., Hu, W., Litthauer, S., Lagarias, J.C., and Harmer, S. (2015). A constitutively active allele of phytochrome B maintains circadian robustness in the absence of light. Plant Physiol. **169**:814–825.

Jung, J.H., Domijan, M., Klose, C., Biswas, S., Ezer, D., Gao, M., Khattak, A.K., Box, M.S., Charoensawan, V., Cortijo, S., et al. (2016). Phytochromes function as thermosensors in *Arabidopsis*. Science **354**:886–889.

Kamioka, M., Takao, S., Suzuki, T., Taki, K., Higashiyama, T., Kinoshita, T., and Nakamichi, N. (2016). Direct repression of evening genes by CIRCADIAN CLOCK-ASSOCIATED1 in the *Arabidopsis* circadian clock. Plant Cell **28**:696–711.

Kiba, T., Henriques, R., Sakakibara, H., and Chua, N.H. (2007). Targeted degradation of PSEUDO-RESPONSE REGULATOR5 by an SCF^{ZTL} complex regulates clock function and photomorphogenesis in *Arabidopsis thaliana*. Plant Cell **19**:2516–2530.

Kidokoro, S., Maruyama, K., Nakashima, K., Imura, Y., Narusaka, Y., Shinwari, Z.K., Osakabe, Y., Fujita, Y., Mizoi, J., Shinozaki, K., et al. (2009). The phytochrome-interacting factor PIF7 negatively regulates DREB1 expression under circadian control in *Arabidopsis*. Plant Physiol. **151**:2046–2057.

Kim, Y.J., and Somers, D.E. (2019). Luciferase-based screen for post-translational control factors in the regulation of the pseudo-response regulator PRR7. Front. Plant Sci. **10**:667.

Kim, J., Yi, H., Choi, G., Shin, B., Song, P.S., and Choi, G. (2003a). Functional characterization of phytochrome interacting factor 3 in phytochrome-mediated light signal transduction. Plant Cell **15**:2399–2407.

Kim, W.Y., Geng, R., and Somers, D.E. (2003b). Circadian phase-specific degradation of the F-box protein ZTL is mediated by the proteasome. Proc. Natl. Acad. Sci. U S A **100**:4933–4938.

Kim, W.Y., Fujiwara, S., Suh, S.S., Kim, J., Kim, Y., Han, L., David, K., Putterill, J., Nam, H.G., and Somers, D.E. (2007). ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. Nature **449**:356–360.

Kim, T.S., Kim, W.Y., Fujiwara, S., Kim, J., Cha, J.Y., Park, J.H., Lee, S.Y., and Somers, D.E. (2011). HSP90 functions in the circadian clock through stabilization of the client F-box protein ZEITLUPE. Proc. Natl. Acad. Sci. U S A **108**:16843–16848.

Kim, J., Geng, R., Gallenstein, R.A., and Somers, D.E. (2013). The F-box protein ZEITLUPE controls stability and nucleocytoplasmic partitioning of GIGANTEA. Development **140**:4060–4069.

Kim, J.Y., Song, J.T., and Seo, H.S. (2017). COP1 regulates plant growth and development in response to light at the post-translational level. J. Exp. Bot. **68**:4737–4748.

Kleine, T., Lockhart, P., and Batschauer, A. (2003). An *Arabidopsis* protein closely related to *Synechocystis* cryptochrome is targeted to organelles. Plant J. **35**:93–103.

Kolmos, E., Herrero, E., Bujdoso, N., Millar, A.J., Tóth, R., Gyula, P., Nagy, F., and Davis, S.J. (2011). A reduced-function allele reveals that *EARLY FLOWERING3* repressive action on the circadian clock is modulated by phytochrome signals in *Arabidopsis*. Plant Cell **23**:3230–3246.

Lau, O.S., and Deng, X.W. (2012). The photomorphogenic repressors COP1 and DET1: 20 years later. Trends Plant Sci. **17**:584–593.

Lau, O.S., Huang, X., Charron, J.B., Lee, J.H., Li, G., and Deng, X.W. (2011). Interaction of *Arabidopsis* DET1 with CCA1 and LHY in mediating transcriptional repression in the plant circadian clock. Mol. Cell **43**:703–712.

Lee, H.G., Mas, P., and Seo, P.J. (2016). MYB96 shapes the circadian gating of ABA signaling in *Arabidopsis*. Sci. Rep. **6**:17754.

Lee, B.D., Kim, M.R., Kang, M.Y., Cha, J.Y., Han, S.H., Nawkar, G.M., Sakuraba, Y., Lee, S.Y., Imaizumi, T., McClung, C.R., et al. (2017). The F-box protein FKF1 inhibits dimerization of COP1 in the control of photoperiodic flowering. Nat. Commun. **8**:2259.

Lee, C.-M., Feke, A., Li, M.-W., Adamchek, C., Webb, K., Pruneda-Paz, J., Bennett, E.J., Kay, S.A., and Gendron, J.M. (2018). Decoys untangle complicated redundancy and reveal targets of circadian clock F-box proteins. Plant Physiol. **177**:1170–1186.

Lee, C.M., Li, M.W., Feke, A., Liu, W., Saffer, A.M., and Gendron, J.M. (2019). GIGANTEA recruits the UBP12 and UBP13 deubiquitylases to regulate accumulation of the ZTL photoreceptor complex. Nat. Commun. **10**:3750.

Legnaioli, T., Cuevas, J., and Mas, P. (2009). TOC1 functions as a molecular switch connecting the circadian clock with plant responses to drought. EMBO J. **28**:3745–3757.

Legris, M., Klose, C., Burgie, E.S., Costigliolo, C., Neme, M., Hiltbrunner, A., Wigge, P.A., Schäfer, E., Vierstra, R.D., and Casal, J.J. (2016). Phytochrome B integrates light and temperature signals in *Arabidopsis*. Science **354**:897–900.

Leivar, P., and Monte, E. (2014). PIFs: systems integrators in plant development. Plant Cell **26**:56–78.

Leivar, P., Monte, E., Al-Sady, B., Carle, C., Storer, A., Alonso, J.M., Ecker, J.R., and Quail, P.H. (2008). The *Arabidopsis* phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels. Plant Cell **20**:337–352.

Leivar, P., Monte, E., Cohn, M.M., and Quail, P.H. (2012). Phytochrome signaling in green *Arabidopsis* seedlings: impact assessment of a mutually negative phyB-PIF feedback loop. Mol. Plant **5**:734–749.

Li, G., Siddiqui, H., Teng, Y., Lin, R., Wan, X.Y., Li, J., Lau, O.S., Ouyang, X., Dai, M., Wan, J., et al. (2011a). Coordinated transcriptional regulation underlying the circadian clock in *Arabidopsis*. Nat. Cell Biol. **13**:616–622.

Li, J., Li, G., Wang, H., and Wang Deng, X. (2011b). Phytochrome signaling mechanisms. *Arabidopsis* Book **9**:e0148.

Li, L., Ljung, K., Breton, G., Schmitz, R.J., Pruneda-Paz, J., Cowing-Zitron, C., Cole, B.J., Ivans, L.J., Pedmale, U.V., Jung, H.-S., et al. (2012). Linking photoreceptor excitation to changes in plant architecture. Genes Dev. **26**:785–790.

Li, X., Ma, D., Lu, S.X., Hu, X., Huang, R., Liang, T., Xu, T., Tobin, E.M., and Liu, H. (2016). Blue light- and low temperature-regulated COR27 and COR28 play roles in the *Arabidopsis* circadian clock. Plant Cell **28**:2755–2769.

Liang, T., Yang, Y., and Liu, H. (2019). Signal transduction mediated by the plant UV-B photoreceptor UVR8. New Phytol. **221**:1247–1252.

Lin, C., Yang, H., Guo, H., Mockler, T., Chen, J., and Cashmore, A.R. (1998). Enhancement of blue-light sensitivity of *Arabidopsis* seedlings by blue light receptor cryptochrome 2. Proc. Natl. Acad. Sci. U S A **95**:2686–2690.

Lin, R., Ding, L., Casola, C., Ripoll, D.R., Feschotte, C., and Wang, H. (2007). Transposase-derived transcription factors regulate light signaling in *Arabidopsis*. Science **318**:1302–1305.

Litthauer, S., Battle, M.W., and Jones, M.A. (2015). Phototropins do not alter accumulation of evening-phased circadian transcripts under blue light. Plant Signal. Behav. **11**:e1126029.

Liu, X.L., Covington, M.F., Fankhauser, C., Chory, J., and Wagner, D.R. (2001). ELF3 encodes a circadian clock-regulated nuclear protein that functions in an *Arabidopsis* PHYB signal transduction pathway. Plant Cell **13**:1293–1304.

Liu, T., Carlsson, J., Takeuchi, T., Newton, L., and Farré, E.M. (2013). Direct regulation of abiotic responses by the *Arabidopsis* circadian clock component PRR7. Plant J. **76**:101–114.

Liu, B., Yang, Z., Gomez, A., Liu, B., Lin, C., and Oka, Y. (2016a). Signaling mechanisms of plant cryptochromes in *Arabidopsis thaliana*. J. Plant Res. **129**:137–148.

Liu, T.L., Newton, L., Liu, M.J., Shiu, S.H., and Farre, E.M. (2016b). A G-box-like motif is necessary for transcriptional regulation by circadian pseudo-response regulators in *Arabidopsis*. Plant Physiol. **170**:528–539.

Liu, Q., Wang, Q., Deng, W., Wang, X., Piao, M., Cai, D., Li, Y., Barshop, W.D., Yu, X., Zhou, T., et al. (2017). Molecular basis for blue light-dependent phosphorylation of *Arabidopsis* cryptochrome 2. Nat. Commun. **8**:15234.

Lu, S.X., Knowles, S.M., Andronis, C., Ong, M.S., and Tobin, E.M. (2009). CIRCADIAN CLOCK ASSOCIATED1 and LATE ELONGATED HYPOCOTYL function synergistically in the circadian clock of *Arabidopsis*. Plant Physiol. **150**:834–843.

Lu, S.X., Webb, C.J., Knowles, S.M., Kim, S.H.J., Wang, Z., and Tobin, E.M. (2012). CCA1 and ELF3 interact in the control of hypocotyl length and flowering time in arabidopsis. Plant Physiol. **158**:1079–1088.

Luo, Q., Lian, H.L., He, S.B., Li, L., Jia, K.P., and Yang, H.Q. (2014). COP1 and phyB physically interact with PIL1 to regulate its stability and photomorphogenic development in *Arabidopsis*. Plant Cell **26**:2441–2456.

Makino, S., Matsushika, A., Kojima, M., Oda, Y., and Mizuno, T. (2001). Light response of the circadian waves of the APRR1/TOC1 quintet: when does the quintet start singing rhythmically in *Arabidopsis*? Plant Cell Physiol. **42**:334–339.

Makino, S., Matsushika, A., Kojima, M., Yamashino, T., and Mizuno, T. (2002). The APRR1/TOC1 quintet implicated in circadian rhythms of *Arabidopsis thaliana*: I. Characterization with APRR1-overexpressing plants. Plant Cell Physiol. **43**:58–69.

Martin-Tryon, E.L., Kreps, J.A., and Harmer, S.L. (2007). GIGANTEA acts in blue light signaling and has biochemically separable roles in circadian clock and flowering time regulation. Plant Physiol. **143**:473–486.

Martín, G., Rovira, A., Veciana, N., Soy, J., Toledo-Ortiz, G., Gommers, C.M.M., Boix, M., Henriques, R., Minguet, E.G., Alabadí, D., et al. (2018). Circadian waves of transcriptional repression shape PIF-regulated photoperiod-responsive growth in *Arabidopsis*. Curr. Biol. **28**:311–318.

Martinez-Garcia, J.F., Huq, E., and Quail, P.H. (2000). Direct targeting of light signals to a promoter element-bound transcription factor. Science **288**:859–863.

Mas, P., Devlin, P.F., Panda, S., and Kay, S.A. (2000). Functional interaction of phytochrome B and cryptochrome 2. Nature **408**:207–211.

Mas, P., Kim, W., Somers, D., and Kay, S.A. (2003). Targeted degradation of TOC1 by ZTL modulates circadian function in *Arabidopsis thaliana*. Nature **426**:567–570.

Mateos, J.L., De Leone, M.J., Torchio, J., Reichel, M., and Staiger, D. (2018). Beyond transcription: fine-tuning of circadian timekeeping by post-transcriptional regulation. Genes **9**:616.

McClung, C.R. (2019). The plant circadian oscillator. Biology **8**:14.

McWatters, H.G., Bastow, R.M., Hall, A., and Millar, A.J. (2000). The ELF3 zeitnehmer regulates light signalling to the circadian clock. Nature **408**:716–720.

Michael, T., Salomé, P., Yu, H., Spencer, T., Sharp, E., McPeek, M., Alonso, J., Ecker, J., and McClung, C. (2003). Enhanced fitness conferred by naturally occurring variation in the circadian clock. Science **302**:1049–1053.

Millar, A.J., and Kay, S.A. (1996). Integration of circadian and phototransduction pathways in the network controlling CAB gene transcription in *Arabidopsis*. Proc. Natl. Acad. Sci. U S A **93**:15491–15496.

Millar, A.J., Straume, M., Chory, J., Chua, N.H., and Kay, S.A. (1995). The regulation of circadian period by phototransduction pathways in *Arabidopsis*. Science **267**:1163–1166.

Mizuno, T., and Nakamichi, N. (2005). Pseudo-response regulators (PRRs) or true oscillator components (TOCs). Plant Cell Physiol. **46**:677–685.

Mizuno, T., Nomoto, Y., Oka, H., Kitayama, M., Takeuchi, A., Tsubouchi, M., and Yamashino, T. (2014a). Ambient temperature signal feeds into the circadian clock transcriptional circuitry through the EC night-time repressor in *Arabidopsis thaliana*. Plant Cell Physiol. **55**:958–976.

Mizuno, T., Takeuchi, A., Nomoto, Y., Nakamichi, N., and Yamashino, T. (2014b). The LNK1 night light-inducible and clock-regulated gene is induced also in response to warm-night through the circadian clock nighttime repressor in *Arabidopsis thaliana*. Plant Signal. Behav. **9**:e28505.

Mombaerts, L., Carignano, A., Robertson, F.C., Hearn, T.J., Junyang, J., Hayden, D., Rutterford, Z., Hotta, C.T., Hubbard, K.E., Maria, M.R.C., et al. (2019). Dynamical differential expression (DyDE) reveals the period control mechanisms of the *Arabidopsis* circadian oscillator. PLoS Comput. Biol. **15**:e1006674.

Monte, E., Tepperman, J.M., Al-Sady, B., Kaczorowski, K.A., Alonso, J.M., Ecker, J.R., Li, X., Zhang, Y., and Quail, P.H. (2004). The phytochrome-interacting transcription factor, PIF3, acts early, selectively, and positively in light-induced chloroplast development. Proc. Natl. Acad. Sci. U S A **101**:16091–16098.

Nagel, D.H., Doherty, C.J., Pruneda-Paz, J.L., Schmitz, R.J., Ecker, J.R., and Kay, S.A. (2015). Genome-wide identification of CCA1 targets uncovers an expanded clock network in *Arabidopsis*. Proc. Natl. Acad. Sci. U S A **112**:E4802–E4810.

Neff, M., and Chory, J. (1998). Genetic interactions between phytochrome A, phytochrome B and cryptochrome 1 during *Arabidopsis* development. Plant Physiol. **118**:27–36.

Ni, M., Tepperman, J.M., and Quail, P.H. (1998). PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein. Cell **95**:657–667.

Ni, W., Xu, S.L., González-Grandío, E., Chalkley, R.J., Huhmer, A.F.R., Burlingame, A.L., Wang, Z.Y., and Quail, P.H. (2017). PPKs mediate direct signal transfer from phytochrome photoreceptors to transcription factor PIF3. Nat. Commun. **8**:15236.

Nieto, C., López-Salmerón, V., Davière, J.-M., and Prat, S. (2015). ELF3-PIF4 interaction regulates plant growth independently of the evening complex. Curr. Biol. **25**:187–193.

Nohales, M.A., and Kay, S.A. (2016). Molecular mechanisms at the core of the plant circadian oscillator. Nat. Struct. Mol. Biol. **23**:1061–1069.

Nohales, M.A., Liu, W., Duffy, T., Nozue, K., Sawa, M., Pruneda-Paz, J.L., Maloof, J.N., Jacobsen, S.E., and Kay, S.A. (2019). Multi-level modulation of light signaling by GIGANTEA regulates both the output and pace of the circadian clock. Dev. Cell **49**:840–851.

Nováková, M., Motyka, V., Dobrev, P.I., Malbeck, J., Gaudinová, A., and Vanková, R. (2005). Diurnal variation of cytokinin, auxin and abscisic acid levels in tobacco leaves. J. Exp. Bot. **56**:2877–2883.

Nozue, K., Covington, M., Duek, P., Lorrain, S., Fankhauser, C., Harmer, S., and Maloof, J. (2007). Rhythmic growth explained by coincidence between internal and external cues. Nature **19**:358–361.

Nusinow, D.A., Helfer, A., Hamilton, E.E., King, J.J., Imaizumi, T., Schultz, T.F., Farre, E.M., and Kay, S.A. (2011). The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature **475**:398–402.

Oakenfull, R.J., and Davis, S.J. (2017). Shining a light on the *Arabidopsis* circadian clock. Plant Cell Environ. **40**:2571–2585.

Oh, E., Zhu, J.-Y., and Wang, Z.-Y. (2012). Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nat. Cell Biol. **14**:802–809.

Paik, I., and Huq, E. (2019). Plant photoreceptors: multi-functional sensory proteins and their signaling networks. Semin. Cell Dev. Biol. **92**:114–121.

Paik, I., Yang, S., and Choi, G. (2012). Phytochrome regulates translation of mRNA in the cytosol. Proc. Natl. Acad. Sci. U S A **109**:1335–1340.

Paik, I., Kathare, P.K., Kim, J.I., and Huq, E. (2017). Expanding roles of PIFs in signal integration from multiple processes. Mol. Plant **10**:1035–1046.

Palágyi, A., Terecskei, K., Ádám, É., Kevei, É., Kircher, S., Mérai, Z., Schäfer, E., Nagy, F., and Kozma-Bognár, L. (2010). Functional analysis of amino-terminal domains of the photoreceptor phytochrome B. Plant Physiol. **153**:1834–1845.

Pedmale, U.V., Huang, S.-S.C., Zander, M., Cole, B.J., Hetzel, J., Ljung, K., Reis, P.A.B., Sridevi, P., Nito, K., Nery, J.R., et al. (2016). Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. Cell **164**:233–245.

Pérez-García, P., Ma, Y., Yanovsky, M.J., and Mas, P. (2015). Time-dependent sequestration of RVE8 by LNK proteins shapes the diurnal oscillation of anthocyanin biosynthesis. Proc. Natl. Acad. Sci. U S A **112**:5249–5253.

Pfeiffer, A., Shi, H., Tepperman, J.M., Zhang, Y., and Quail, P.H. (2014). Combinatorial complexity in a transcriptionally centered signaling hub in *Arabidopsis*. Mol. Plant **7**:1598–1618.

Pham, V.N., Kathare, P.K., and Huq, E. (2018a). Phytochromes and phytochrome interacting factors. Plant Physiol. **176**:1025–1038.

Pham, V.N., Xu, X., and Huq, E. (2018b). Molecular bases for the constitutive photomorphogenic phenotypes in *Arabidopsis*. Development **145**:dev169870.

Podolec, R., and Ulm, R. (2018). Photoreceptor-mediated regulation of the COP1/SPA E3 ubiquitin ligase. Curr. Opin. Plant Biol. **45**:18–25.

Pokhilko, A., Fernandez, A.P., Edwards, K.D., Southern, M.M., Halliday, K.J., and Millar, A.J. (2012). The clock gene circuit in *Arabidopsis* includes a repressilator with additional feedback loops. Mol. Syst. Biol. **8**:574.

Pruneda-Paz, J.L., Breton, G., Para, A., and Kay, S.A. (2009). A functional genomics approach reveals CHE as a component of the *Arabidopsis* circadian clock. Science **323**:1481–1485.

Pudasaini, A., Shim, J.S., Song, Y.H., Shi, H., Kiba, T., Somers, D.E., Imaizumi, T., and Zoltowski, B.D. (2017). Kinetics of the LOV domain of ZEITLUPE determine its circadian function in *Arabidopsis*. eLife **6**:e21646.

Pudasaini, A., and Zoltowski, B.D. (2013). Zeitlupe senses blue-light fluence to mediate circadian timing in *Arabidopsis thaliana*. Biochemistry **52**:7150–7158.

Ramos-Sánchez, J.M., Triozzi, P.M., Alique, D., Geng, F., Gao, M., Jaeger, K.E., Wigge, P.A., Allona, I., and Perales, M. (2019). LHY2 integrates night-length information to determine timing of poplar photoperiodic growth. Curr. Biol. **29**:2402–2406.

Rawat, R., Takahashi, N., Hsu, P.Y., Jones, M.A., Schwartz, J., Salemi, M.R., Phinney, B.S., and Harmer, S.L. (2011). REVEILLE8 and PSEUDO-REPONSE REGULATOR5 form a negative feedback loop within the *Arabidopsis* circadian clock. PLoS Genet. **7**:e1001350.

Reed, J.W., Nagatani, A., Elich, T.D., Fagan, M., and Chory, J. (1994). Phytochrome A and phytochrome B have overlapping but distinct functions in *Arabidopsis* development. Plant Physiol. **104**:1139–1149.

Reed, J.W., Nagpal, P., Bastow, R.M., Solomon, K.S., Dowson-Day, M.J., Elumalai, R.P., and Millar, A.J. (2000). Independent action of ELF3 and phyB to control hypocotyl elongation and flowering time. Plant Physiol. **122**:1149–1160.

Rizzini, L., Favory, J.-J., Cloix, C., Faggionato, D., O'Hara, A., Kaiserli, E., Baumeister, R., Schäfer, E., Nagy, F., Jenkins, G.I., et al. (2011). Perception of UV-B by the *Arabidopsis* UVR8 protein. Science **332**:103–106.

Rizzini, L., Levine, D.C., Perelis, M., Bass, J., Peek, C.B., and Pagano, M. (2019). Cryptochromes-mediated inhibition of the $CRL4^{Cop1}$-complex assembly defines an evolutionary conserved signaling mechanism. Curr. Biol. **29**:1954–1962.

Rugnone, M.L., Faigón Soverna, A., Sanchez, S.E., Schlaen, R.G., Hernando, C.E., Seymour, D.K., Mancini, E., Chernomoretz, A., Weigel, D., Más, P., et al. (2013). LNK genes integrate light and clock signaling networks at the core of the *Arabidopsis* oscillator. Proc. Natl. Acad. Sci. U S A **110**:12120–12125.

Salomé, P.A., and McClung, C.R. (2005). PSEUDO-RESPONSE REGULATOR 7 and 9 are partially redundant genes essential for the temperature responsiveness of the *Arabidopsis* circadian clock. Plant Cell **17**:791–803.

Salomé, P.A., Michael, T.P., Kearns, E.V., Fett-Neto, A.G., Sharrock, R., and McClung, R.C. (2002). The out of phase 1 mutant defines a role for PHYB in circadian phase control in *Arabidopsis*. Plant Physiol. **129**:1674–1685.

Sanchez, S.E., and Kay, S.A. (2016). The plant circadian clock: from a simple timekeeper to a complex developmental manager. Cold Spring Harb. Perspect. Biol. **8**:a027748.

Sawa, M., Nusinow, D., Kay, S., and Imaizumi, T. (2007). FKF1 and GIGANTEA complex formation is required for day-length measurement in *Arabidopsis*. Science **318**:261–265.

Schäfer, E., and Nagy, F. (2006). Photomorphogenesis in Plants and Bacteria: Function and Signal Transduction Mechanisms (Dordrecht: Springer).

Schaffer, R., Ramsay, N., Samach, A., Corden, S., Putterill, J., Carré, I.A., and Coupland, G. (1998). The late elongated hypocotyl mutation of arabidopsis disrupts circadian rhythms and the photoperiodic control of flowering. Cell **93**:1219–1229.

Schaffer, R., Landgraf, J., Accerbi, M., Simon, V., Larson, M., and Wisman, E. (2001). Microarray analysis of diurnal and circadian-regulated genes in *Arabidopsis*. Plant Cell **13**:113–123.

Schultz, T.F., Kiyosue, T., Yanovsky, M., Wada, M., and Kay, S.A. (2001). A role for LKP2 in the circadian clock of *Arabidopsis*. Plant Cell **13**:2659–2670.

Seaton, D.D., Toledo-Ortiz, G., Ganpudi, A., Kubota, A., Imaizumi, T., and Halliday, K.J. (2018). Dawn and photoperiod sensing by phytochrome A. Proc. Natl. Acad. Sci. U S A **115**:10523–10528.

Seo, P.J., and Mas, P. (2015). STRESSing the role of the plant circadian clock. Trends Plant Sci. **20**:230–237.

Sharrock, R.A., and Clack, T. (2002). Patterns of expression and normalized levels of the five *Arabidopsis* phytochromes. Plant Physiol. **130**:442–456.

Shikata, H., Hanada, K., Ushijima, T., Nakashima, M., Suzuki, Y., and Matsushita, T. (2014). Phytochrome controls alternative splicing to mediate light responses in *Arabidopsis*. Proc. Natl. Acad. Sci. U S A **111**:18781–18786.

Shor, E., Paik, I., Kangisser, S., Green, R., and Huq, E. (2017). PHYTOCHROME INTERACTING FACTORS mediate metabolic control of the circadian system in *Arabidopsis*. New Phytol. **215**:217–228.

Singh, M., and Mas, P. (2018). A functional connection between the circadian clock and hormonal timing in *Arabidopsis*. Genes **9**:567.

Somers, D.E., Devlin, P.F., and Kay, S.A. (1998). Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian clock. Science **282**:1488–1490.

Somers, D.E., Kim, W.Y., and Geng, R. (2004). The F-box protein ZEITLUPE confers dosage-dependent control on the circadian clock, photomorphogenesis, and flowering time. Plant Cell **16**:769–782.

Song, Y.H., Shim, J.S., Kinmonth-Schultz, H.A., and Imaizumi, T. (2015). Photoperiodic flowering: time measurement mechanisms in leaves. Annu. Rev. Plant Biol. **66**:441–464.

Soy, J., Leivar, P., González-Schain, N., Martín, G., Diaz, C., Sentandreu, M., Al-Sady, B., Quail, P.H., and Monte, E. (2016). Molecular convergence of clock and photosensory pathways through PIF3-TOC1 interaction and co-occupancy of target promoters. Proc. Natl. Acad. Sci. U S A **113**:4870–4875.

Strasser, B., Sánchez-Lamas, M., Yanovsky, M.J., Casal, J.J., and Cerdán, P.D. (2010). *Arabidopsis thaliana* life without phytochromes. Proc. Natl. Acad. Sci. U S A **107**:4776–4781.

Takase, T., Nishiyama, Y., Tanihigashi, H., Ogura, Y., Miyazaki, Y., Yamada, Y., and Kiyosue, T. (2011). LOV KELCH PROTEIN2 and ZEITLUPE repress *Arabidopsis* photoperiodic flowering under non-inductive conditions, dependent on FLAVIN-BINDING KELCH REPEAT F-BOX1. Plant J. **67**:608–621.

Takeuchi, T., Newton, L., Burkhardt, A., Mason, S., and Farré, E.M. (2014). Light and the circadian clock mediate time-specific changes in sensitivity to UV-B stress under light/dark cycles. J. Exp. Bot. **65**:6003–6012.

Tepperman, J., Zhu, T., Chang, H., Wang, X., and Quail, P. (2001). Multiple transcription-factor genes are early targets of phytochrome A signaling. Proc. Natl. Acad. Sci. U S A **98**:9437–9442.

Thain, S.C., Vandenbussche, F., Laarhoven, L.J.J., Dowson-Day, M.J., Wang, Z.-Y., Tobin, E.M., Harren, F.J.M., Millar, A.J., and Van Der Straeten, D. (2004). Circadian rhythms of ethylene emission in *Arabidopsis*. Plant Physiol. **136**:3751–3761.

Thines, B., and Harmon, F.G. (2010). Ambient temperature response establishes ELF3 as a required component of the core *Arabidopsis* circadian clock. Proc. Natl. Acad. Sci. U S A **107**:3257–3262.

Tóth, R., Kevei, E., Hall, A., Millar, A., Nagy, F., and Kozma-Bognár, L. (2001). Circadian clock-regulated expression of phytochrome and cryptochrome genes in *Arabidopsis*. Plant Physiol. **127**:1607–1616.

Ushijima, T., Hanada, K., Gotoh, E., Yamori, W., Kodama, Y., Tanaka, H., Kusano, M., Fukushima, A., Tokizawa, M., Yamamoto, Y.Y., et al. (2017). Light controls protein localization through phytochrome-mediated alternative promoter selection. Cell **171**:1316–1325.

Viczián, A., Kircher, S., Fejes, E., Millar, A.J., Schäfer, E., Kozma-Bognár, L., and Nagy, F. (2005). Functional characterization of phytochrome interacting factor 3 for the *Arabidopsis thaliana* circadian clockwork. Plant Cell Physiol. **46**:1591–1602.

Wang, Z.Y., and Tobin, E.M. (1998). Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) gene disrupts circadian rhythms and suppresses its own expression. Cell **93**:1207–1217.

Wang, H., and Wang, H. (2015). Phytochrome signaling: time to tighten up the loose ends. Mol. Plant **8**:540–551.

Wang, Y., Wu, J.-F., Nakamichi, N., Sakakibara, H., Nam, H.-G., and Wu, S.-H. (2011). LIGHT-REGULATED WD1 and PSEUDO-RESPONSE REGULATOR9 form a positive feedback regulatory loop in the *Arabidopsis* circadian clock. Plant Cell **23**:486–498.

Webb, A.A.R., Seki, M., Satake, A., and Caldana, C. (2019). Continuous dynamic adjustment of the plant circadian oscillator. Nat. Commun. **10**:550.

Wenden, B., Kozma-Bognár, L., Edwards, K.D., Hall, A.J.W., Locke, J.C.W., and Millar, A.J. (2011). Light inputs shape the *Arabidopsis* circadian system. Plant J. **66**:480–491.

Whitelam, G.C., Johnson, E., Peng, J., Carol, P., Anderson, M.L., Cowl, J.S., and Harberd, N.P. (1993). Phytochrome A null mutants of *Arabidopsis* display a wild-type phenotype in white light. Plant Cell **5**:757–768.

Wu, J.-F., Tsai, H.-L., Joanito, I., Wu, Y.-C., Chang, C.-W., Li, Y.-H., Wang, Y., Hong, J.C., Chu, J.-W., Hsu, C.-P., et al. (2016). LWD-TCP complex activates the morning gene CCA1 in *Arabidopsis*. Nat. Commun. **7**:13181.

Xie, Q., Wang, P., Liu, X., Yuan, L., Wang, L., Zhang, C., Li, Y., Xing, H., Zhi, L., Yue, Z., et al. (2014). LNK1 and LNK2 are transcriptional coactivators in the *Arabidopsis* circadian oscillator. Plant Cell **26**:2843–2857.

Yakir, E., Hilman, D., Kron, I., Hassidim, M., Melamed-Book, N., and Green, R.M. (2009). Posttranslational regulation of CIRCADIAN CLOCK ASSOCIATED1 in the circadian oscillator of *Arabidopsis*. Plant Physiol. **150**:844–857.

Yamashino, T., Matsushika, A., Fujimori, T., Sato, S., Kato, T., Tabata, S., and Mizuno, T. (2003). A link between circadian-trolled bHLH factors and the APRR1/TOC1 quintet in *Arabidopsis thaliana*. Plant Cell Physiol. **44**:619–629.

Yang, P., Wang, J., Huang, F.-Y., Yang, S., and Wu, K. (2018). The plant circadian clock and chromatin modifications. Genes **9**:561.

Yang, Z., Liu, B., Su, J., Liao, J., Lin, C., and Oka, Y. (2017). Cryptochromes orchestrate transcription regulation of diverse blue light responses in plants. Photochem. Photobiol. **93**:112–127.

Yanovsky, M.J., Izaguirre, M., Wagmaister, J.A., Gatz, C., Jackson, S.D., Thomas, B., and Casal, J.J. (2000a). Phytochrome A resets the circadian clock and delays tuber formation under long days in potato. Plant J. **23**:223–232.

Yanovsky, M.J., Mazzella, M.A., and Casal, J.J. (2000b). A quadruple photoreceptor mutant still keeps track of time. Curr. Biol. **10**:1013–1015.

Yanovsky, M.J., Whitelam, G.C., and Casal, J.J. (2000c). *fhy3-1* retains inductive responses of phytochrome A. Plant Physiol. **123**:235–242.

Yanovsky, M.J., Mazzella, M.A., Whitelam, G.C., and Casal, J.J. (2001). Resetting of the circadian clock by phytochromes and cryptochromes in *Arabidopsis*. J. Biol. Rhythms **16**:523–530.

Yasuhara, M., Mitsui, S., Hirano, H., Takanabe, R., Tokioka, Y., Ihara, N., Komatsu, A., Seki, M., Shinozaki, K., and Kiyosue, T. (2004). Identification of ASK and clock-associated proteins as molecular partners of LKP2 (LOV kelch protein 2) in *Arabidopsis*. J. Exp. Bot. **55**:2015–2027.

Yeom, M., Kim, H., Lim, J., Shin, A.-Y., Hong, S., Kim, J.-I., and Nam, H.G. (2014). How do phytochromes transmit the light quality information to the circadian clock in *Arabidopsis*? Mol. Plant **7**:1701–1704.

Yin, R., and Ulm, R. (2017). How plants cope with UV-B: from perception to response. Curr. Opin. Plant Biol. **37**:42–48.

Yu, J.-W., Rubio, V., Lee, N.-Y., Bai, S., Lee, S.-Y., Kim, S.-S., Liu, L., Zhang, Y., Irigoyen, M.L., Sullivan, J.A., et al. (2008). COP1 and ELF3 control circadian function and photoperiodic flowering by regulating GI stability. Mol. Cell **32**:617–630.

Yu, X., Liu, H., Klejnot, J., and Lin, C. (2010). The cryptochrome blue light receptors. *Arabidopsis* Book **8**:e0135.

Zagotta, M.T., Hicks, K.A., Jacobs, C.I., Young, J.C., Hangarter, R.P., and Meeks-Wagner, D.R. (1996). The *Arabidopsis ELF3* gene regulates vegetative photomorphogenesis and the photoperiodic induction of flowering. Plant J. **10**:691–702.

Zeugner, A., Byrdin, M., Bouly, J.-P., Bakrim, N., Giovani, B., Brettel, K., and Ahmad, M. (2005). Light-induced electron transfer in *Arabidopsis* cryptochrome-1 correlates with in vivo function. J. Biol. Chem. **280**:19437–19440.

Zhang, Y., Mayba, O., Pfeiffer, A., Shi, H., Tepperman, J.M., Speed, T.P., and Quail, P.H. (2013). A quartet of PIF bHLH factors provides a transcriptionally centered signaling hub that regulates seedling morphogenesis through differential expression-patterning of shared target genes in *Arabidopsis*. PLoS Genet. **9**:e1003244.

Zhang, C., Gao, M., Seitz, N.C., Angel, W., Hallworth, A., Wiratan, L., Darwish, O., Alkharouf, N., Dawit, T., Lin, D., et al. (2019a). LUX ARRHYTHMO mediates crosstalk between the circadian clock and defense in *Arabidopsis*. Nat. Commun. **10**:2543.

Zhang, Y., Bo, C., and Wang, L. (2019b). Novel crosstalks between circadian clock and jasmonic acid pathway finely coordinate the tradeoff among plant growth, senescence and defense. Int. J. Mol. Sci. **20**:5254.

Zhang, Y., Pfeiffer, A., Tepperman, J.M., Dalton-Roesler, J., Leivar, P., Gonzalez Grandio, E., and Quail, P.H. (2020). Central clock components modulate plant shade avoidance by directly repressing transcriptional activation activity of PIF proteins. Proc. Natl. Acad. Sci. U S A https://doi.org/10.1073/pnas.1918317117.

Zhao, X., Jiang, Y., Li, J., Huq, E., Chen, Z.J., Xu, D., and Deng, X.W. (2018). COP1 SUPPRESSOR 4 promotes seedling photomorphogenesis by repressing *CCA1* and *PIF4* expression in *Arabidopsis*. Proc. Natl. Acad. Sci. U S A **115**:11631–11636.

Zhong, H.H., Painter, J.E., Salomé, P.P., Straume, M., and McClung, R. (1998). Imbibition, but not release from stratification, sets the circadian clock in *Arabidopsis* seedlings. Plant Cell **10**:2005–2017.

Zhu, J.-Y., Oh, E., Wang, T., and Wang, Z.-Y. (2016). TOC1-PIF4 interaction mediates the circadian gating of thermoresponsive growth in *Arabidopsis*. Nat. Commun. **7**:13692.