Gearing Up the Clock of Hypocotyl Growth!

To anticipate daily changes imposed by Earth's rotation, organisms from all domains of life have evolved an endogenous 24-h timer with daily rhythmic functions: the circadian clock. In plants, the first organisms where circadian dynamics were observed, the circadian clock processes daylength information to regulate a wide range of physiological and developmental responses (Greenham and McClung, 2015). Under normal photoperiodic conditions, hypocotyl elongation is a robust response that can be observed to monitor the circadian regulation of plant growth (Nusinow et al., 2011). However, the regulatory networks controlling this physiological mechanism remain poorly understood.

In this issue of *Plant Physiology*, Li et al. (2020) approach this topic by demonstrating coordinated action of specific components of the Arabidopsis (*Arabidopsis thaliana*) circadian clock to regulate photoperiodic hypocotyl growth (Li et al., 2020). They focused on the Evening Complex (EC), which is composed of EARLY FLOWERING4 (ELF4), ELF3, and LUX ARRHYTHMO; and the Pseudo Response Regulators (PRRs) gene family composed of five members (PRR3, PRR5, PRR7, PRR9, and TIMING OF CAB EXPRESSION1 [TOC1]), all components of the circadian clock. One of the functions of the EC and PRRs in plant photoperiodic growth dynamics is to regulate the abundance and activity of PHYTOCHROME-INTERACTING FACTOR (PIF) transcription factors, which are positive regulators of plant cell elongation (Nusinow et al., 2011; Martin et al., 2018). Previous work has demonstrated that, whereas EC components (ELF3, ELF4, and LUX ARRHYTHMO) inhibit *PIF* expression in the early evening and the beginning of the night (thus directly allowing the circadian clock to diurnally regulate hypocotyl growth), PRR proteins (PRR3, PRR5, PRR7, PRR9, and TOC1) are proposed to modulate photoperiodic hypocotyl elongation via transcriptional regulation and post-transcriptional control of *PIF4* and *PIF5* (Liu et al., 2013; Martin et al., 2018). Because PIF proteins promote cell elongation, any conditions that promote PIF expression and accumulation, such as short days and high temperature, induce long hypocotyl phenotypes.

Li et al. (2020) used a series of higher-order mutants to evaluate the genetic interactions of PRRs and EC components on hypocotyl length upon different light regimes (short day, long day, and continuous light). They found that the hypocotyls of *toc1 prr5 elf3* and *toc1 prr5 lx* triple mutants were significantly longer than those of lower-order mutants, both in short day and long day, suggesting that PRR and EC additively regulate this growth response under photoperiodic conditions. Remarkably, continuous light conditions abolished any hypocotyl growth difference indicating that daylight cycling is required to reveal the role of clock genes on hypocotyl growth.

How do the EC and PRRs additively control PIF and what is the impact on the hypocotyl elongation? To address this, the authors carried out a transcriptomic study of various PRR mutants, which revealed that PRRs regulate *PIF* expression, as *PIFs* have been previously shown to be transcriptional targets of the EC. Previous studies have indicated that TOC1 protein also interacts with PIFs through direct protein–protein interactions. Here, the authors demonstrated that although the protein–protein interaction occurs independently of TOC1's DNA binding domain, TOC1 requires its DNA binding domain to rescue a *toc1* knockout mutant. These data support an important transcriptional role for TOC1 in regulating PIF expression levels and hypocotyl growth. The authors also showed that TOC1/PRR5s are able to directly bind *PIF* promoter regions and repress them.

More important, the authors showed that the circadian expression of *PIF4* and *PIF5* is cooperatively orchestrated by PRRs and EC acting at slightly different times of the evening, with PRRs repressing PIFs expression earlier than EC components. Using the *toc1 prr5 elf3* triple mutant, the authors showed that daylength imposes dynamic changes on the expression of *PIF4* and *PIF5*, depending on the presence of specific PRRs and EC. In doing so, PRRs and EC proteins adjust daylength information by affecting the cyclic expression of *PIFs*. Additionally, the *toc1prr5 pif4 pif5* quadruple mutant showed that PRR-mediated clock regulation of hypocotyl elongation requires a functional PIF4 and PIF5 and hence PIF4 and PIF5 act downstream of PRRs. In other words, the coordination of the hypocotyl-growth response mediated by PIF4 and PIF5 is synchronized with daylength by the clock elements PRRs and EC.

Molecular characterization of the plant circadian clock is helping us to understand how plants regulate a plethora of daily rhythmic physiological events. As interconnected mechanical gears that control the functioning of a true physical clock, authors demonstrated that EC and PRR work additively to regulate circadian dynamics of photoperiodic hypocotyl growth. Given that hypocotyl growth is critical for the success of seedling establishment, work by Li et al. (2020) may allow us to understand the physiological processes controlling seedling establishment and plant survival in natural conditions. In this regard, synchrony between the external light–dark cycle and the internal plant clock results in improved growth and survival

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parameters (Green et al., 2002; Dodd et al., 2005). Besides photoperiodic growth, the described mechanism of work for the PRRs-EC-PIF signaling module may serve as a tool to explore other clock-modulated responses in plants, such as metabolites accumulation (Dodd et al., 2015), foraging for light (Salter et al., 2003) and defense against pests and pathogens (Goodspeed et al., 2013). The clock keeps ticking and the time to move circadian research into the wild is gearing up!

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LITERATURE CITED

Dodd AN, Belbin FE, Frank A, Webb AA (2015) Interactions between circadian clocks and photosynthesis for the temporal and spatial coordination of metabolism. Front Plant Sci **6:** 245

Dodd AN, Salathia N, Hall A, Kévei E, Tóth R, Nagy F, Hibberd JM, Millar AJ, Webb AA (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science **309:** 630–633

Goodspeed D, Chehab EW, Covington MF, Braam J (2013) Circadian control of jasmonates and salicylates: The clock role in plant defense. Plant Signal Behav **8:** e23123

Green RM, Tingay S, Wang ZY, Tobin EM (2002) Circadian rhythms confer a higher level of fitness to Arabidopsis plants. Plant Physiol **129:** 576–584

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

Li N, Zhang Y, He Y, Wang Y, Wang L (2020) Pseudo response regulators regulate photoperiodic hypocotyl growth by repressing PIF4/5 transcription. Plant Physiol **183:** 686–699

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

Martin G, Rovira A, Veciana N, Soy J, Toledo-Ortiz G, Gommers CMM, Boix M, Henriques R, Minguet EG, Alabadi D, et al (2018) Circadian waves of transcriptional repression shape PIF-regulated photoperiod-responsive growth in Arabidopsis. Curr Biol **28:** 311–318 e315

Nusinow DA, Helfer A, Hamilton EE, King JJ, Imaizumi T, Schultz TF, Farré EM, Kay SA (2011) The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature **475:** 398–402

Salter MG, Franklin KA, Whitelam GC (2003) Gating of the rapid shade-avoidance response by the circadian clock in plants. Nature **426:** 680–683