



Focus on circadian rhythms

Stacey L. Harmer ^{1,*} Christian Fankhauser ² and Alex A. R. Webb ³

1 Department of Plant Biology, University of California, One Shields Avenue, Davis, California 95616, USA

2 Faculty of Biology and Medicine, Centre for Integrative Genomics, University of Lausanne, Génopode Building, Lausanne CH-1015, Switzerland

3 Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK

*Author for correspondence: slharmer@ucdavis.edu

Editorial

In the 24 years since the first plant circadian oscillator genes were cloned (Schaffer et al., 1998; Wang and Tobin, 1998), there has been a concerted effort to identify the mechanistic basis of circadian rhythms and to understand how the circadian system impacts the biology of plants. Progress has been so great that there is now a good understanding of the circadian biology of *Arabidopsis* and insight into the circadian biology of the major crops is developing. For this Focus Issue on Circadian Rhythms, we encouraged submission of articles that emphasize less understood aspects, such as interactions between the circadian system and the natural environment, cell-specific function, setting of the circadian clock by environmental signals, and evolution of plant circadian oscillators.

The *Arabidopsis* circadian oscillator was first modeled as a simple negative feedback loop, with two Myb-like transcription factors, CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), directly inhibiting expression of *TIMING OF CAB EXPRESSION 1* (TOC1). TOC1, a member of the PSEUDO-RESPONSE REGULATOR family, was first proposed to promote CCA1 and LHY expression (Alabadi et al., 2001). However, later studies revealed that TOC1 directly represses CCA1 and LHY expression (Gendron et al., 2012; Huang et al., 2012; Pokhilko et al., 2012), with these three genes forming a double negative feedback loop. Subsequent work by many laboratories has revealed that the circadian oscillator mechanism in *Arabidopsis* is instead actually composed of dozens of genes that regulate each other's expression, forming a very complex interconnected network (Nakamichi, 2020). Post-translational regulation of circadian proteins is also pervasive, enhancing the robustness of the circadian system and fine-tuning circadian period (Yan et al., 2021).

In addition to controlling each other's expression, this slew of circadian oscillator transcription factors regulate a

large fraction of the plant transcriptome, with up to 40% of *Arabidopsis* genes reported to be under circadian regulation (Romanowski et al., 2020). Given the widespread influence of circadian oscillators on gene expression, it is not surprising that many aspects of physiology, including photosynthesis, abiotic and biotic stress responses, and the transition from vegetative to reproductive growth are modulated by the circadian system (Sanchez and Kay, 2016). Indeed, breeding during domestication has selected for allelic variants in circadian clock genes in a large number of crop species (Steed et al., 2021; Maeda and Nakamichi, 2022). Future improvements in crop germplasm and agronomic practices will depend upon a better understanding of the circadian system and its interactions with the environment.

While circadian clocks are present in most living organisms, they have independently arisen in different lineages (Jabbur and Johnson, 2022). In this issue, Peterson et al. provide an Update on recent advances in understanding the evolution of circadian components in the green lineage. Genomic surveys indicate deep conservation of core elements of circadian oscillators, not only in land plants but also in Chlorophyte and Charophyte algae. Moreover, Peterson and colleagues summarize our current understanding of the function of circadian components in several land plants and algae (Petersen et al., 2022). Maeda and Nakamichi (2022) provide an excellent Update covering human selection of varieties suited for local conditions (including photoperiod and other climatic features) which allowed the cultivation of plants outside the ecological range of their wild ancestors. These authors present examples from long-day plants, such as wheat and barley, and short-day plants, such as rice and sorghum. How this process led to the selection of alleles that either flower early, or late, to avoid particularly dry, or wet times of the year, is well illustrated (Maeda and Nakamichi, 2022). Greenham et al. (2022)

contribute an Update that discusses the adaptive nature of circadian clocks more broadly. This Update describes the complex interrelationship between temperature and light cycles that organisms must cope with both on a daily and a seasonal basis. The authors also emphasize the importance of performing more studies in natural conditions due to the difficulties of properly mimicking such conditions in controlled environments (Greenham et al., 2022). Close genetic linkage between some circadian oscillator and photosensing genes might be important for robust circadian function and appropriate growth in specific natural environments. In an analysis of chromosome-resolved plant genomes and syntenic orthologs, Todd Michael found high conservation of specific genetic linkages across most angiosperms and he suggests these linkages might predate the land plants (Michael, 2022). These linkages may help promote the inheritance of a robust and site-appropriate circadian system in both inbreeding and outbreeding species.

Adjusting circadian timing in response to environment signals in processes summarized as entrainment is an important aspect of circadian biology, because while the oscillator generates the rhythms, it is entrainment that ensures that internal events occur at the right time of day to ensure beneficial outcomes. Wang et al. (2022) update the latest advances in understanding how the Arabidopsis circadian oscillator entrains to the cycles of light and dark, and warm and cold, that are part of the daily experience for plants and other organisms in the wild. Additionally, Wang et al. (2022) describe the molecular players recently found to be involved in entrainment to internal metabolic cycles. These authors conclude that the basic processes of entrainment are relatively poorly understood because of the complexity of the problem and because the network architecture of the circadian oscillator changes over time. Further insight into entrainment mechanisms might be aided by formal description of the processes in mathematical models. New clues about potential mechanisms by which light information is transmitted to the circadian oscillator are provided by experimental research findings in this issue. Ronald et al. (2022) report that both red and blue light affect the localization of the key circadian oscillator protein ELF3 in nuclear foci, with the former process mediated by phytochrome B-dependent and -independent pathways. The downstream effects of changes in the sub-nuclear localization of ELF3 remain unclear and will no doubt be the topic of future research.

Cell-type differences in entrainment pathways could explain the spatial differences in circadian timing found across the plant body, as described in an Update by Davis et al. (2022). In considering the spatial aspects of circadian timing, Davis et al. (2022) describe how new single cell-type sequencing and imaging innovations have revealed that circadian oscillators in different cell types can run at different speeds and that in some tissues communication between neighboring cells coordinates circadian timing through weak coupling. While entrainment effects might explain why

circadian oscillators in different tissues have different behaviors, it is also possible that the network properties of the oscillators vary between cell types. Differences in network properties might also account for the different dynamics in different tissues. Davis and colleagues conclude that to fully understand the mechanism and purpose of cell-type differences in circadian timing, the full extent of coupling needs to be resolved and there needs to be a fuller characterization of the extent of the differences in circadian timing across cell types.

It is the regulation of circadian clock outputs shaping plant physiology and development that give meaning to the intricacies of circadian regulation. Scandola et al. (2022) used transcriptomics, proteomics, and metabolomics to determine how the core circadian oscillator Myb-like REVEILLE (RVE) transcription factors affect plant physiology. They made the surprising finding that not only are critical elements of primary metabolism altered in plants mutant for *rve4 rve6 rve8*, but that these plants have reduced levels of subunits of the 20S core proteasome and impaired proteasome activity. This reduced proteasome activity may help explain the previously reported enhanced growth phenotype of *rve4 rve6 rve8* mutants (Gray et al., 2017). In another investigation of links between core oscillator components and plant physiology, Wei et al. (2022) use whole-genome approaches to identify direct targets of the Myb-like circadian oscillator protein CCA1 in rice. They report that CCA1 regulates abscisic acid (ABA) signaling to affect stress responses through its role as a transcriptional regulator of many output genes, including those involved in ABA signaling. The importance of this regulatory role in stress responses of rice is indicated by their discovery of reduced survival in loss-of-function lines in response to NaCl stress. Indeed, the circadian oscillator has an important role in modulating plant responses to a range of environmental stresses (Panter et al., 2019). One such stress is high temperature. In this issue, Bonnot et al. (2022) present a tool, CAST-R, that allows the easy visualization of circadian and heat stress-responsive genes. Using CAST-R, users can: dynamically visualize connections between selected circadian proteins and their output genes; generate diel and circadian profiles of transcript abundance from Arabidopsis, *Brassica rapa*, barley, and rice; and perform phase enrichment analysis for both user-supplied and previously published rhythmic datasets. This tool provides an excellent resource for scientists interested in understanding linkages between the circadian system and plant environmental responses.

In closing, the updates and research articles presented in this issue reflect both the profound influence of the circadian system on plant physiology and the impressive progress made in this field over the past few decades. We thank the authors, editors, and reviewers for efforts that have contributed to the papers that are collected in the Focus Issue and will be associated over the coming months.

Conflict of interest statement. None declared.

References

- Alabadi D, Oyama T, Yanovsky MJ, Harmon FG, Mas P, Kay SA** (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the Arabidopsis circadian clock. *Science* **293**: 880–883
- Bonnot T, Gillard MB, Nagel DH** (2022) CAST-R: An application to visualize circadian and heat stress-responsive genes in plants. *Plant Physiol* **190**: 994–1004
- Davis W, Endo M, Locke JCW** (2022) Spatially specific mechanisms and functions of the plant circadian clock. *Plant Physiol* **190**: 938–951
- Gendron JM, Pruneda-Paz JL, Doherty CJ, Gross AM, Kang SE, Kay SA** (2012) Arabidopsis circadian clock protein, TOC1, is a DNA-binding transcription factor. *Proc Natl Acad Sci USA* **109**: 3167–3172
- Gray JA, Shalit-Kaneh A, Chu DN, Hsu PY, Harmer SL** (2017) The REVEILLE clock genes inhibit growth of juvenile and adult plants by control of cell size. *Plant Physiol* **173**: 2308–2322
- Greenham K, Oravec MW** (2022) The adaptive nature of the plant circadian clock in natural environments. *Plant Physiol* **190**: 968–980
- Huang W, Perez-Garcia P, Pokhilko A, Millar AJ, Antoshechkin I, Riechmann JL, Mas P** (2012) Mapping the core of the Arabidopsis circadian clock defines the network structure of the oscillator. *Science* **336**: 75–79
- Jabbur ML, Johnson CH** (2022) Spectres of clock evolution: past, present, and yet to come. *Front Physiol* **12**: 815847
- Maeda AE, Nakamichi N** (2022) Plant clock modifications for adapting flowering time to local environments. *Plant Physiol* **190**: 952–967
- Michael TP** (2022) Core circadian clock and light signaling genes brought into genetic linkage across the green lineage. *Plant Physiol* **190**: 1037–1056
- Nakamichi N** (2020) The transcriptional network in the Arabidopsis circadian clock system. *Genes* **11**: 1284
- Panter PE, Muranaka T, Cuitun-Coronado D, Graham CA, Yochikawa A, Kudoh H, Dodd AN** (2019) Circadian regulation of the plant transcriptome under natural conditions. *Front Genet* **10**: 1239
- Petersen J, Rredhi A, Szyttenholm J, Mittag M** (2022) Evolution of circadian clocks along the green lineage. *Plant Physiol* **190**: 924–937
- Pokhilko A, Fernández AP, Edwards KD, Southern MM, Halliday KJ, Millar AJ, Fernandez AP, Edwards KD, Southern MM, Halliday KJ, et al.** (2012) The clock gene circuit in Arabidopsis includes a repressor with additional feedback loops. *Mol Syst Biol* **8**: 574
- Romanowski A, Schlaen RG, Perez-Santangelo S, Mancini E, Yanovsky MJ** (2020) Global transcriptome analysis reveals circadian control of splicing events in *Arabidopsis thaliana*. *Plant J* **103**: 889–902
- Ronald J, Su C, Wang L, Davis SJ** (2022) Cellular localization of Arabidopsis EARLY FLOWERING3 is responsive to light quality. *Plant Physiol* **190**: 1024–1036
- Sanchez SE, Kay SA** (2016) The plant circadian clock: from a simple timekeeper to a complex developmental manager. *Cold Spring Harb Perspect Biol* **8**: a027748. doi:10.1101/cshperspect
- Scandola S, Mehta D, Li Q, Rodriguez Gallo MC, Castillo B, Uhrig RG** (2022) Multi-omic analysis shows REVEILLE clock genes are involved in carbohydrate metabolism and proteasome function. *Plant Physiol* **190**: 1005–1023
- Schaffer R, Ramsay N, Samach A, Corden S, Putterill J, Carre IA, Coupland G, Carré IA, Coupland G** (1998) The late elongated hypocotyl mutation of Arabidopsis disrupts circadian rhythms and the photoperiodic control of flowering. *Cell* **93**: 1219–1229
- Steed G, Ramirez DC, Hannah MA, Webb AAR** (2021) Chronoculture, harnessing the circadian clock to improve crop yield and sustainability. *Science* **372**: eabc9141
- Wang S, Steed G, Webb AAR** (2022) Circadian entrainment in Arabidopsis. *Plant Physiol* **190**: 981–993
- Wang Z-YY, Tobin EM** (1998) Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. *Cell* **93**: 1207–1217
- Wei H, Xu H, Su C, Wang X, Wang L** (2022) Rice CIRCADIAN CLOCK ASSOCIATED1 transcriptionally regulates ABA signaling to confer multiple abiotic stress tolerance. *Plant Physiol* **190**: 1057–1073
- Yan J, Kim YJ, Somers DE** (2021) Post-translational mechanisms of plant circadian regulation. *Genes* **12**: 325