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REVIEW

Making the clock tick: the transcriptional landscape of the plant circadian clock [version 1; referees: 2 approved]

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Abstract
Circadian clocks are molecular timekeepers that synchronise internal physiological processes with the external environment by integrating light and temperature stimuli. As in other eukaryotic organisms, circadian rhythms in plants are largely generated by an array of nuclear transcriptional regulators and associated co-regulators that are arranged into a series of interconnected molecular loops. These transcriptional regulators recruit chromatin-modifying enzymes that adjust the structure of the nucleosome to promote or inhibit DNA accessibility and thus guide transcription rates. In this review, we discuss the recent advances made in understanding the architecture of the Arabidopsis oscillator and the chromatin dynamics that regulate the generation of rhythmic patterns of gene expression within the circadian clock.

Open Peer Review

Invited Referees

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Introduction
The daily rotation of the Earth generates predictable diurnal changes in light and temperature. Circadian clocks act as endogenous timekeepers to coordinate internal physiological responses to match the predicted environmental condition. The plant circadian clock directly regulates a range of output pathways, which includes hormone signalling, hypocotyl development, metabolism, the floral transition, photosynthesis, and the response to biotic and abiotic stress. Accordingly, plants with an internal clock that matches the external environment (~24 hours) have enhanced photosynthesis and survival compared to plants with a clock that does not match the external environment.

Endogenous circadian rhythms are generated through a series of interconnected transcriptional–translational feedback loops, collectively termed the oscillator. Light and temperature signals differentially converge on the plant oscillator through multiple input pathways to provide timing cues in a process termed entrainment. In plants, light signals at dawn are thought to act as the major entraining signal. This review will discuss the recent advances made in understanding the transcriptional architecture of the plant oscillator and the chromatin dynamics driving rhythmic gene expression.

Overview of the oscillator
At the core of the plant oscillator are the morning-expressed, partially redundant MYB domain transcription factors (TFs) CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYOCOTYL (LHY). CCA1/LHY directly antagonise most clock gene expression through binding to the evening element (EE) motif within the promoter. One target of CCA1/LHY-repressive activity is the PSEUDO-RESPONSE REGULATOR (PRR) TIMING OF CAB EXPRESSION (TOC1). CCA1/LHY-repressive activity restricts TOC1 expression to a window around dusk. At dusk, TOC1 accumulates and reciprocally represses CCA1/LHY expression in addition to other clock genes. This mutual antagonism between CCA1/LHY and TOC1 defines the central loop of the Arabidopsis oscillator.

Additional interconnected loops subsequently regulate the activity of the core loop. At dawn, the TFs TEOSINTE BRANCHED CYCLOIDEA-PCF20/22 (TCP20/22) recruit the co-activator LIGHT REGULATED WD 1 (LWD1) to activate CCA1/LHY expression. LWD1 and its homolog LWD2 are also required to activate the expression of TOC1 and the related PRR5, PRR7, and PRR9. PRR5, PRR7, and PRR9 directly associate with the CCA1/LHY promoter and repress CCA1/LHY expression. PRR9, PRR7, and PRR5 are sequentially expressed, generating a wave of repressive activity. PRR9 expression starts at dawn, followed by PRR7 in the late morning and PRR5 in the afternoon. This repressive sequence is re-enforced by the CCA1-related MYB TF REVEILLET8 (RVE8) and its associated homologs, RVE6 and RVE4. RVE8 binds to the EE within the PRR5, TOC1, and EARLY FLOWERING 4 (ELF4) promoter and activates gene expression by recruiting the co-activators NIGHT LIGHT-INDUCIBLE AND CLOCK REGULATED 1/2 (LNK1/LNK2). In the evening, the GARP TF LUX ARRHYTHMO (LUX) and the unrelated proteins ELF3 and ELF4 associate to form the evening complex (EC). The EC represses the morning-expressed PRR7 and PRR9 and evening-expressed GIGANTEA (GI) and LUX. LUX and ELF3 have also been recently shown to associate with the promoter of LNK1/2, highlighting another potential target of the EC. Together, this interconnected network of activators and repressors drives rhythmic gene expression within the plant oscillator.

Chromatin dynamics of the circadian clock
The structure of nucleosomes has a fundamental role in regulating gene expression. A nucleosome is a complex of DNA wound around the histone octamer (two H2A-H2B dimers and a H3-H4 tetramer). Each histone unit can be post-translationally modified through a suite of chromatin-remodelling enzymes to generate what is collectively called the histone code. These modifications regulate the accessibility of the DNA through opening or compacting the histone octamer or by providing a binding site for other chromatin-modifying enzymes. Modifications associated with transcriptional activation include the acetylation of H3 lysine residues (H3Ac) or tri-methylation of H3K4me3, while repressive markers include the tri-methylation of H3K9 (H3K9me3) and H3K27 (H3K27me3).
Other chromatin-remodelling enzymes have also been associated with the plant oscillator. The histone acetyltransferase (HAT) TAF1 and the HDA HD1 regulates the acetylation and de-acetylation of the TOC1 and CCA1 promoter, respectively. However, TAF1 and HDA1 loss-of-function mutants had only a small effect on TOC1 and CCA1 expression. Arabidopsis has 12 HATs and 18 HDAs, and within each respective class functional redundancy has been observed. HATs and HDAs are therefore likely to be acting redundantly within the clock. Alongside HATs and HDAs, histone methylases and demethylases have also been implicated within the clock. The H3K4me3 methylase SET DOMAIN GROUP 2 (SDG2/ATRX3) aids clock gene expression and the ability of TOC1 to associate with DNA. The histone demethylase JUMONJI DOMAIN CONTAINING 5 (JMJD5), also referred to as JMJD30, displays diurnal regulation with expression peaking in the evening. JMJD5 mutants have a shortening of circadian period, suggesting that JMJD5 has a regulatory role within the oscillator. Remarkably, Arabidopsis JMJD5 has retained conserved functional activity with its human orthologue, which functions within the mammalian circadian clock. However, the mammalian JMJD5 lacks canonical demethylase activity. Further work is needed to understand the functional activity of JMJD5 and its role within the Arabidopsis clock. It has also been recently shown that 17 different chromatin-remodelling enzymes display diurnal patterns of expression, further intertwining the relationship between the clock and chromatin remodellers. In summary, the concerted activities of a broad range of histone-modifying enzymes are required within the clock to facilitate the transcriptional regulatory activity of the plant oscillator.

Conclusions and perspectives

In recent years, much progress has been made in connecting the individual components of the oscillator into an interconnected transcriptional network. However, many questions still persist over the mechanisms of transcriptional regulation. The association of RVE8 to the TOC1 promoter correlates with hyperacetylation, but neither RVE8 nor LNK1/2 have domains that could recruit HAT directly. The repressive mechanisms of the core components CCA1/LHY and TOC1 are also poorly understood. TOC1 has been recently shown to co-occupy PHYTOCHROME INTERACTING FACTOR 3 (PIF3) target promoters and inhibit PIF3-mediated gene activation. However, whether this is achieved by passively inhibiting HAT recruitment or by actively recruiting co-repressors through an unidentified repression domain is unknown. It also remains unclear whether CCA1/LHY repress gene expression passively or actively, with both mechanisms being proposed in a temporal-dependent manner. Furthermore, CCA1 and LHY are often grouped together and viewed as a joint operator within the clock. However, CCA1 and LHY have been shown to have distinct roles within the clock. Future work could investigate the extent of functional overlap between CCA1/LHY.

One noticeable shortage in the plant clock when compared to the mammalian or fungal circadian clock are transcriptional activators. CCA1/LHY, TOC1, and the plant-specific protein GI were all proposed to act as transcriptional activators within the oscillator. However, these have now been shown to be an indirect relationship or an effect caused by the mutant background used. In eukaryotes, the default state of gene expression is often one of a repressive nature, so transcriptional activators would be expected within the oscillator.

The discovery of the RVE8/LNKs and the TCP/LWD complex has provided some answers to the mechanisms of transcriptional activation within the oscillator. However, recent mathematical modelling of the oscillator that incorporated RVE8 has shown a non-reliance of the oscillator on transcriptional activation. The activation of the oscillator genes could be sourced externally. The transcript induction of CCA1, LHY, GI, PRR9, PRR7, LNK1, LNK2, ELF3, and ELF4 are all positively regulated by light. Additionally, the expression of LUX, PRR7, and PRR9 is activated in a temperature-dependent manner. Thus, external environmental signals may participate in gene activation within the clock, while the repressive circuitry of the clock acts to antagonise and attenuate these external gene activation pathways. What is notable in this is the finding that a large proportion of transcription factors are rhythmic and a subset of those can modulate clock parameters. Together, it appears that known activators within the clock act to fine-tune prevailing environmental antagonism as a form of signal integration.

Transcriptional regulators and the associated chromatin landscape governing transcriptional regulation are only one level nestled within a multi-layered regulatory network. Post-translational modicfications, nucleocytoplasmic partitioning, RNA splicing, and protein degradation all have their own essential role in aiding rhythm generation. It is only through the integration of all of these layers of activity that the plant clock can generate and sustain robust rhythms and facilitate the response to diurnal changes in the environment.

Competing interests

The authors declare that they have no competing interests.

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Version 1

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