

Recent advances in computational modeling as a conduit to understand the plant circadian clock

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Abstract

The circadian clock is necessary for plants to anticipate environmental changes. This leads to a coordination of plant development and growth and thus to increased fitness. Many clock components were identified by genetic and biochemical approaches, and studies on these components revealed a core oscillator with multiple feedback loops. A suite of computation analyses is uncovering the outputs of this oscillating network. Mathematical analysis is contributing to our understanding of the network under clock control, moving toward an explanation of how the clock integrates and coordinates various developmental programs with daily environmental cues. From there, these systems approaches will look to establish further nodes within the clock network.

Introduction and context

The earth is continuously rotating and this generates repetitive day-night changes each day. As sessile organisms, plants perceive various environmental signals and adjust to these changes. Thus, predictable day-night changes are important signals for plants to synchronize an internal biological clock to the external time of day, and this creates the useful circadian regulatory mechanism.

The plant circadian network can be conceived as being composed of an input pathway, a core oscillator, and output pathways [1]. Plants recognize light quality, quantity, and duration, and variations in temperature as input signals. These predictable environmental signals have been termed “zeitgebers,” as they are the cues that lead to clock resetting in response to seasonal photo-period differences – a process known as entrainment. The core oscillator has been shown to consist of multiple autoregulatory feedback loops, and to date, many *Arabidopsis thaliana* clock genes have been identified [1]. Several core clock proteins are transcription factors and are additionally proposed to initiate the mechanism

of the clock by generating widespread transcriptional rhythms [2]. For instance, the clock-output pathways potentiate various biological responses, such as development, metabolism, hormone responses, and photo-periodic response [1,3]. At a molecular level, the expression of approximately 30% of the total transcriptome of *A. thaliana* has been found to be under circadian regulation [4].

The *A. thaliana* clock has been especially well defined regarding components that generate a transcriptional feedback loop. Two morning elements, LATE ELONGATED HYPOCOTYL (LHY) and CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1), are partially redundant MYB-like transcription factors and repress transcription of the evening-acting factor *TIMING OF CAB EXPRESSION 1* (*TOC1*) by direct binding to its promoter evening-element region. Conversely, *TOC1* promotes *LHY* and *CCA1* transcription and consequently forms a negative feedback loop, and this has been classified as the core of the oscillator [5]. However, this one-loop model was not sufficient to explain various mutant phenotypes. For example, the *lhy cca1* double mutant and the *toc1* single

mutant display short periodicity rather than arrhythmicity. In addition, the *lhy cca1* double mutant is entrainable by light-dark cycles [6-8]. Moreover, through intensive genetic investigations, additional clock-regulating components were identified. TIME FOR COFFEE (TIC), PSEUDO-RESPONSE REGULATOR 7 (PRR7), and PRR9 act in the morning, and GIGANTEA (GI), EARLY FLOWERING 3 (ELF3), ELF4, and LUX ARRHYTHMO (LUX) function in the evening. Mutations of these genes lead to strong phenotypic perturbations within the clock. Therefore, the functional position of these components in the clock model was questioned and the need to make a more inclusive clock model was reinforced [9-15].

Computational analysis was introduced as one systems approach to assist the understanding of the molecular genetics of the plant clock. This allowed for a mathematical examination of the robustness of the clock upon the quantitative changes of various parameters. Several parameters were calculated to 'set up' the mathematical circadian-clock model, such as light as an input signal, transcription and mRNA degradation rates of the clock genes, protein turnover rates, and protein transport [16]. The first mathematically established multiple-loop *A. thaliana* clock model incorporated two undefined components as X and Y. Although genes acting as an X have not yet been identified, its transcription was mathematically predicted to be increased by the genetic activity of TOC1. From there, X promotes the transcription of *LHY/CCA1*. Y was stated to act to form another loop with *LHY/CCA1* and TOC1. Both *LHY/CCA1* and TOC1 repress the expression of Y, whereas Y was postulated to promote *TOC1* transcription. Y was simulated to be acutely upregulated by light. In this work, *GI* was suggested as a partial component of Y [17].

Major recent advances

Since the creation of the two-loop interlocking model could not explain the short-period phenotype of the *toc1* mutant [6], and since this model had limited flexibility to simulate photoperiodic changes, the combination of newly identified experimental results with computational analysis was used to expand the clock as a three-loop circuit. It adopted PRR7 and PRR9 to form an additional morning loop with *LHY/CCA1*. *LHY/CCA1* positively regulated the expression of *PRR7* and *PRR9*, and *PRR7* and *PRR9* repressed *LHY* and *CCA1* expression [11,13,18]. The factor X, from above, was now placed as a connection between the *LHY/CCA1-PRR7/PRR9* morning loop and the *TOC1-Y* evening loop [19,20]. These models provided explicit and testable hypotheses, which could largely be confirmed by genetic approaches [21]. Other uses of the three-loop model were to computationally predict the

placement of *ELF4* within the oscillating network and to predict how an arrhythmic circadian mutant could exist as a single-gene, loss-of-function allele [22]. Collectively, systems modeling provided a gene-regulatory circuit that explains much of the molecular genetic data in the literature [22].

Recent mathematical studies have widened our scope of understanding from the simple core-clock oscillation to more complex responses, such as the perception of a seasonal variation or flowering-time regulation. A predictive photoperiodic pathway has been mathematically demonstrated [23]. Moreover, a view of how the oscillator is buffered from unpredictable environmental changes, such as daily weather patterns, can be rationalized through modeling approaches [24].

Future directions

As a companion with experimental approaches, systems modeling has widened our understanding of the *A. thaliana* circadian clock. Furthermore, structural modeling is expanding the predictive scope of inferred biochemistry of clock proteins [22,25]. In spite of these breakthroughs, there are still a number of questions to be resolved. First, parameters were oversimplified in most mathematical models. For example, *LHY* and *CCA1* have been treated as if they were one component [19,20], although they are only partially redundant [8,26,27]. Second, various input factors should be considered. In current mathematical models, light was the only input factor included in the circadian clock. However, there are other factors, such as temperature, humidity, metabolism, and hormone responses, and these must be incorporated in the model to explain entrainment [28,29]. Third, post-transcriptional regulation of the clock proteins should be more seriously considered. Recently, several post-translational modifications, including phosphorylation of *TOC1* and protein-protein interactions within the various PRRs, were hypothesized to be an important regulatory mechanism of the core clock. Nevertheless, these biochemical interactions have not yet been incorporated into the network model [30,31]. Fourth, in addition to nuclear signaling, a metabolic clock should be considered. This is because it has been reported that the cytosolic Ca^{2+} signaling molecule cADPR (cyclic adenosine diphosphate ribose) could be mathematically incorporated into the circadian clock [32]. Finally, regulation of output pathways affected by the core clock should be incorporated into various computational models. For example, multiple developmental processes, such as flowering and photomorphogenesis, have been experimentally revealed to be under the circadian-clock regulation [1,3,21]. How to build the exact regulatory structure from the core

clock to output responses must be clarified, and systems modeling through computational approaches will clearly aid that need.

Abbreviations

CCA1, CIRCADIAN CLOCK-ASSOCIATED 1; ELF, EARLY FLOWERING; GI, GIGANTEA; LHY, LATE ELONGATED HYPOCOTYL; PRR, PSEUDO-RESPONSE REGULATOR; TOC1, TIMING OF CAB EXPRESSION 1.

Competing interests

The authors declare that they have no competing interests.

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