4-9-2018

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Kai Wang
Louisiana State University

John C. Larkin
Louisiana State University

Recommended Citation
Wang, K., & Larkin, J. (2018). A Tale of Two Cycles. *Developmental Cell, 45* (1), 8-9. https://doi.org/10.1016/j.devcel.2018.03.016

A Tale of Two Cycles

Kai Wang[1] and John C. Larkin[1,*]
[1]Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA
*Correspondence: jlarkin@lsu.edu
https://doi.org/10.1016/j.devcel.2018.03.016

Two of the fundamental rhythms of eukaryotic life are the circadian clock and the cell division cycle. In this issue of *Developmental Cell*, Fung-Uceda and colleagues (2018) have elucidated a molecular mechanism linking the circadian clock to the cell cycle in the plant *Arabidopsis thaliana*.

Living organisms are replete with cycles of recurring events, at levels ranging from metabolism to behavior. Two of the most fundamental cycles in eukaryotic life are the circadian clock, which allows organisms to synchronize their activities with the daily light-dark cycle, and the cell division cycle, which drives cellular reproduction. Both of these cycles are maintained by interlocked feedback loops operating at both transcriptional and posttranscriptional levels. In the case of the circadian clock in plants, two core clock transcription factors, CCA and LHY, transcriptionally repress members of the *PRR* family in the morning, and *PRR* genes are successively expressed as the day progresses (Nohales and Kay, 2016). The last member of the *PRR* family to be expressed is the key clock regulator *TOC1*, which is expressed in the afternoon and prevents premature activation of an "evening complex" of regulators, as well as inhibiting expression of earlier clock genes. The phases of the plant cell cycle are driven forward by the action of cyclin-dependent kinases (CDKs), and successive cell-cycle stages are terminated by a combination of cyclin-dependent kinase inhibitors (KRPs and SMRs) and ubiquitin-mediated proteolysis (Polyn et al., 2015). Entry into S phase, when DNA is replicated, is one of the most highly regulated events in the cell cycle. A prerequisite for DNA replication is binding of CDC6 to replication origins, which can only occur during G1, when CDK activity is at its lowest (Castellano et al., 2004; Brasil et al., 2017). However, DNA replication cannot begin until CDK levels rise at the G1/S transition, thus preventing re-replication of origins after S has begun. In this issue of *Developmental Cell*, Fung-Uceda et al. (2018) demonstrate a direct link between *TOC1* and *CDC6*, linking the circadian clock to the cell cycle (Figure 1).

The starting point for this work was the observation by Fung-Uceda and colleagues (2018) that plants constitutively overexpressing the clock regulator *TOC1* (TOC1-ox) had a dwarf phenotype. Leaf area, cell area, and epidermal cell numbers in TOC-ox plants were all considerably reduced compared to wild-type plants, as was the rate of cell division in leaves. Flow cytometry indicated that G1 was longer and S phase was shorter relative to wild-type. After exiting mitosis, many *Arabidopsis* leaf cells continue to replicate their DNA without subsequent division, a process known as endoreplication (De Veylder et al., 2011), and Fung-Uceda et al. (2018) found that TOC1-ox also reduced the degree of endoreplication. On the other hand, a *toc1-2* mutant showed larger leaf size with increased cell number, as well as enhanced endoreplication, and similar results were obtained by RNAi.

Examination of the expression patterns of a number of cell-cycle genes revealed significant differences between TOC-ox and wild-type plants, both in the timing of expression during leaf development and, most significantly, during the diurnal cycle. Genes showing altered expression patterns included D-type cyclins, members of both the *KRP* and *SMR* families of CKIs, and the *CDH1* homolog *CCS52A2*. The two genes that in wild-type were expressed early during the light period, *SMR5* and *CDC6*, were strongly repressed in TOC-ox plants regardless of time of day. Further, when Fung-Uceda and colleagues (2018) examined the expression of

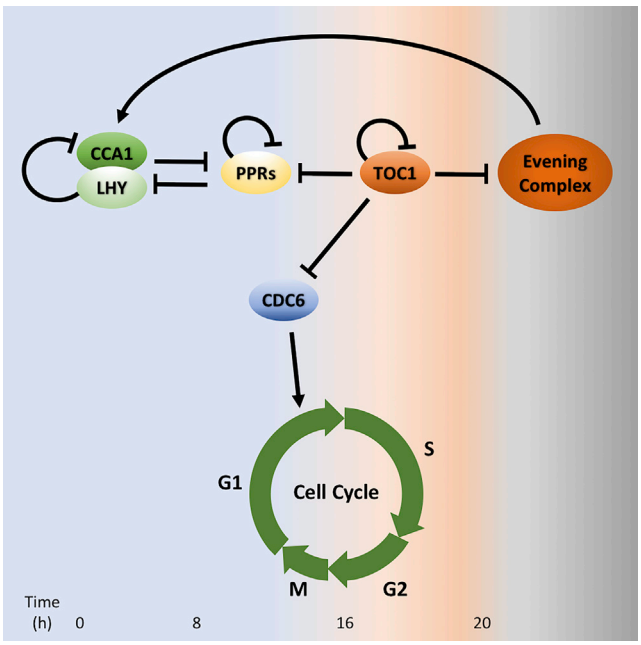

Figure 1. A Direct Connection between the Circadian Clock and the Cell Cycle
The top of the figure shows a highly simplified schematic of the circadian clock. Diurnal time starts with dawn at the left, and clock components are arranged roughly in the order of their function. The phases of the cell division cycle are shown in the bottom portion of the diagram. Fung-Uceda et al. (2018) have demonstrated that TOC1, a circadian clock protein whose function peaks in the afternoon, is a direct repressor of *CDC6* transcription. Binding of the CDC6 protein to replication origins is a prerequisite for DNA replication, and thus CDC6 is an important regulator of entry into S phase of the cell cycle.

both *CDC6* and *TOC1* in wild-type, they found that the peak expression patterns during diurnal cycles were out of phase with each other, suggesting that *TOC1* is antagonistic to *CDC6* expression. The authors then showed by chromatin immunoprecipitation that TOC1 binds to the *CDC6* promoter, but not to the promoters of several other cell-cycle genes, and that in wild-type plants, TOC1 binding to the *CDC6* promoter is maximal late in the light period, when *TOC1* transcripts are most abundant. Finally, using a transient expression assay in protoplasts, the authors show that TOC1 can repress expression of a reporter gene from the *CDC6* promoter but cannot repress expression from mutant versions of the *CDC6* promoter in which the "evening element," a known TOC1 binding site, has been altered. Taken together, these lines of evidence convincingly demonstrate that TOC1 directly represses *CDC6* expression.

The circadian clock of plants coordinates a wide variety of plant responses to the environment (Greenham and McClung, 2015). Fung-Uceda et al. (2018) have now demonstrated a direct connection between the circadian clock and another crucial clock-like aspect of plant growth, the cell cycle. One striking implication noted by the authors is that via this connection, the circadian clock might synchronize the cell cycle with the diurnal cycle, with S phase most likely to occur early in the light period, prior to the peak of TOC1 expression. The close link between these two important cycles should perhaps not be such a surprise. Indeed, cell division in the green algae *Chlamydomonas reinhardtii*, which has many cell-cycle features in common with Angiosperms, is very strictly regulated by the photoperiod, with growth occurring during an extended G1 phase during the light period, followed in the dark by several rapid S/M phases without an intervening G2 to produce multiple daughter cells (Cross and Umen, 2015). It is interesting to note that the transcription factor TCP20 is necessary for transcription of *CCA1* at dawn. TCP20 has also been implicated in the regulation of an *Arabidopsis* mitotic cyclin (Li et al., 2005), hinting that there may be other links between the two cycles. Finally, the work of Fung-Uceda et al. (2018) may have direct implications for agriculture. The work that they have presented shows that altering the links between the circadian clock and the cell cycle can affect leaf size, suggesting that manipulating this link could potentially increase biomass and crop yields. For example, Guo and colleagues have shown that mutants increasing cell number in maize can directly impact both biomass and yield in a major crop plant (Guo et al., 2010). It is very likely that further exploration of links between the circadian clock and the cell cycle will be fruitful.

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