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Review

Transcriptional versus non-transcriptional clocks: A case study in *Ostreococcus*

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ABSTRACT

Circadian rhythms are ubiquitous on earth from cyanobacteria to land plants and animals. Circadian clocks are synchronized to the day/night cycle by environmental factors such as light and temperature. In eukaryotes, clocks rely on complex gene regulatory networks involving transcriptional regulation but also post-transcriptional and post-translational regulations. In multicellular organisms clocks are found at multiple levels from cells to organs and whole organisms, making the study of clock mechanisms more complex. In recent years the picoalga *Ostreococcus* has emerged as a new circadian model organism thanks to its reduced gene redundancy and its minimalist cellular organization. A simplified version of the "green" plant clock, involving the master clock genes *TOC1* and *CCA1*, has been revealed when the functional genomics and mathematical model approaches were combined.
Specific photoreceptors such as a blue light sensing LOV histidine kinase mediate light input to the *Ostreococcus* clock. Non-transcriptional redox rhythms have also been identified recently in *Ostreococcus* and human red blood cells. This review highlights the progress made recently in the understanding of circadian clock architecture and function in *Ostreococcus* in the context of the marine environment.

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1. Introduction

Most living organisms are exposed to changes in light and temperature in a 24 hour period due to the rotation of the earth around its axis. Light is the main source of energy for photosynthetic organisms which

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constitute the basis of food chains in terrestrial and oceanic environments. Light, however, can be detrimental to cells as UV radiation damages genetic material. Living organisms have evolved endogenous clocks, called circadian clocks, which allow them to best benefit from the 24 hour day/night cycle. In photosynthetic organisms, for example, the transcription of photosynthesis proteins is under circadian control and begins before dawn so that photosynthesis can start as soon as light appears (Harmer and Kay, 2000; Monnier et al., 2010). As well as anticipating periodic environmental changes the circadian clock orchestrates biological processes during the day/night cycle thus avoiding incompatible processes occurring at the same time. During the cell division cycle DNA should not be exposed to reactive oxygen species or damaging UV light both of which can induce mutations. Circadian regulation of cell division has been evidenced in several microalgae including *Ostreococcus* and *Chlamydomonas* (Moulager et al., 2007; Goto and Johnson, 1995). Furthermore, circadian rhythms of survival to UV radiation have been observed in *Chlamydomonas*, the cells being most vulnerable at the time of nuclear division (Nikaido and Johnson, 2000). This suggests that light-sensitive processes are phased along the day/night cycle to avoid sunlight induced damage.

Although the earth rotation period is constant from day to day, the relative day to night length, the photoperiod, varies during the year, the winter/summer differences being more pronounced at high latitudes. A key feature of circadian clocks is their ability to be entrained to a wide range of photoperiods. This feature is called flexibility. The clock, however, must also be resistant (or robust) to noisy light input not linked to earth rotation. These light fluctuations usually arise from weather conditions (e.g. clouds in the sky) or factors that alter the water column mixing for phytoplanktonic microorganisms. The circadian clock can also be entrained by temperature cycles, but its free-running period is less sensitive to temperature variations so that seasonal changes in temperature have little effect on circadian rhythms. Last but not the least, persistence under free running conditions with a period of ~24 h is the main property of circadian rhythms.

Circadian rhythms have been observed at the cellular level in animals, plants and bacteria. The emergence of genetic approaches in model organisms has enabled scientists to identify first clock mutants (e.g. long-period, short-period of even arrhythmic), then clock genes. Twenty years after the initial genetic studies in model organisms, the molecular clock architecture of the circadian clock is well established in several organisms including *Drosophila* (insects), mice (mammals), *Neurospora* (fungi) and *Arabidopsis* (plants). The nature of circadian rhythms, however, is complex in multicellular organisms, since rhythms and underlying clocks are observed not only at the cellular level but also at the organ and whole organism levels. Furthermore these clocks can be uncoupled, for example during jetlag. This adds an additional level of complexity when studying the circadian clock at the molecular level.

2. *Ostreococcus* a new circadian model organism

In recent years the green picoalga *Ostreococcus tauri* has emerged as a new model organism for functional genomics and systems biology approaches. This minimalist cell of only 1 μm in diameter has only one chloroplast and a single mitochondrion in addition to the nucleus. The genome of only 12.6 Mb (the size of *Saccharomyces cerevisiae* genome) is the most compact of known eukaryotic genomes with intergenic regions smaller than 200 bp (Derelle et al., 2006). The gene families are extremely reduced with very little gene redundancy facilitating the use of reverse genetic approaches. Molecular tools based on genetic transformation, such as gene functional analysis by overexpression/knockdown or luciferase reporter fusions have been used to monitor circadian rhythms and analyze the function.

Besides the molecular tools described above, *Ostreococcus* has many advantages for circadian studies. Cells can be easily synchronized with the day/night cycle. Under 12:12 light/dark cycles, most biological processes are temporally orchestrated. In *Ostreococcus*, Bayesian Fourier clustering analysis has revealed clusters of co-regulated genes involved in specific biological processes expressed rhythmic of candidate genes (Corellou et al., 2009; Moulager et al., 2010; Djouani-Tahri et al., 2011a; Heijde et al., 2010) along the day/night cycle. The extent of this is unprecedented when compared to other eukaryotes. (Monnier et al., 2010). This genome-wide transcriptomic study of gene expression indicates that transcriptional regulations play a key role in the diurnal/circadian regulation of gene expression. During the night genes involved in general transcription, ribosome synthesis and translation are successively transcribed. From dawn to dusk, clusters are enriched in genes involved in photosynthesis, response to UV stress (midday), DNA replication and finally, cell division at the end of the day (Moulager et al., 2007; Monnier et al., 2010). Rhythms of cell division and the transcription of key cell cycle regulators persist under constant light, consistent with a circadian control of cell division (Corellou et al., 2009; Djouani-Tahri et al., 2011a; Moulager et al., 2007; Heijde et al., 2010). Cell cycle related proteins such as dynamin and kinesin were also found to be upregulated during the daylight-to-darkness transition in shotgun proteomic analysis (Le Bihan et al., 2011).

3. A simple transcriptional clock

3.1. A two-gene oscillator

The overall architecture of transcriptional circadian clocks is conserved between kingdoms although the main molecular players are different. The ~24 hour rhythm results from transcriptional translational feedback loops in which clock components activate the synthesis of their own repressors. Synthetic biology is able to reproduce rhythms based on simple genetic circuits such as the represillator in bacteria (Elowitz and Leibler, 2000). The resulting oscillations, however, are noisy and the periods much shorter than 24 h. Delays resulting from translational regulation, protein degradation, post-translational modifications and subcellular localization of clock components allow the circadian period to extend to ~24 h in mathematical models. Although a simple oscillator should be sufficient to generate a rhythm, the clocks of model organisms such as *Drosophila* or *Arabidopsis* have been shown to rely on multiple coupled loops (e.g. morning and evening loops) which allow more flexibility (Troein et al., 2009).

Extensive searches have been performed in silico to identify putative conserved clock components in *Ostreococcus*, taking advantage of the sequenced genome. Only two putative candidates with homology to Time of CAB expression1 (TOC1) and Circadian Clock associated1 (CCA1) transcription factors have been identified. These were also the first two clock components identified in plants (Strayer et al., 2000; Green and Tobin, 1999). It was proposed initially that TOC1 and CCA1 interact in a simple negative feedback loop in which CCA1 represses the transcription of *TOC1*,TOC1 TOC1 activates the synthesis of *CCA1* (Alabadí et al., 2001). However *TOC1* transcription is thought to begin once CCA1 has been degraded. This model has been challenged further by genetic analysis and modeling approaches (reviewed in Carré and Veflingstad, 2013). The current outline of the *Arabidopsis* clock relies on three coupled loops, and unlike the above model, TOC1 is a repressor of *CCA1* transcription (Huang et al., 2012; Gendron et al., 2012). In *Ostreococcus* CCA1 has been shown to bind in vitro a perfectly conserved AAAATATCT evening element motif, found in the promoter of *TOC1*, which is required for circadian expression of *TOC1* (Corellou et al., 2009). Overexpression of CCA1 leads to a downregulation of *TOC1* consistent with a repressing activity of CCA1. CCA1 repression by antisense had no effect on circadian rhythms of TOC1 in constant light. However, it caused aberrant patterns of rhythmic expression under 6:6 cycles, suggesting altered circadian regulation of TOC1 responses to light. This suggests that either CCA1 repression was not sufficient to destabilize the TOC1/CCA1 loop in the antisense lines studied or that

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