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Proteasome Function Is Required for Biological Timing throughout the Twenty-Four Hour Cycle

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depends crucially on the interplay between fitness landscape and population dynamics. Given the complex fitness landscape observed, the size and mutation rate of the evolving population set the limits for second-order selection of clones with increased evolvability. Had the population been smaller, the *spoT* mutation that rescued the EW clone might not have occurred before the clone went extinct, turning the clone into an 'eventual loser' instead of the topic of a research project. As the authors mention, a minimal requirement for second-order selection of evolvability is the simultaneous presence of multiple contending clones carrying different beneficial mutations. Beyond that, population size and mutation rate determine how far evolution can look into the future, that is, how many new beneficial mutations are allowed to accumulate before the EW clone fixes. These results support previous claims that there is ample opportunity for higher-order selection of evolvability in microbial populations, since often multiple beneficial mutations accumulate before they fix [10].

Several other studies have found evidence for the complexity of real fitness landscapes [11–14], and for the importance of population dynamic parameters for adaptation on these landscapes. For instance, it was found that small bacterial populations sometimes reached higher fitness than populations 50-fold larger in size, despite their lower fitness early on [15]. These results were explained by assuming that large populations adapt by using bigger-effect mutations — those that survive the

competition [16] — which would sometimes lead to local maxima. Small populations use different mutations each time, some of which would lead to higher fitness maxima, particularly when steep slopes lead to low peaks [17]. A recent study with the enzyme β-lactamase found that alternative initial mutations repeatedly directed adaptation onto different mutational pathways [18]. Here, drift — the chance occurrence of the first mutation — was again important for evolvability, but it was the mutation with greatest benefit that directed evolution to a higher peak.

The study by Woods *et al.* [2] is about selection for evolvability over relatively short time scales, allowing a single fixation event. However, selection for increased evolvability may also happen at longer time scales involving multiple selective sweeps, but then as a result from competition between rather than within populations. Future studies should address the factors determining long-term evolvability, for which Woods *et al.* [2] provide an important framework, conceptually as well as methodologically.

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Laboratory of Genetics, Wageningen University, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands.
*E-mail: Arjan.devisser@wur.nl

Circadian Rhythms: Lost in Post-Translation

Multiple studies question the necessity of transcription/translation feedback loops for the generation of circadian rhythms. New data emphasize the necessity of proteasomal degradation for circadian rhythmicity in transcriptionally competent cells.

C. Robertson McClung

Circadian rhythms, endogenous rhythms with periods of approximately 24 hours, have been described in almost all organisms, from cyanobacteria to humans. These rhythms are the products of endogenous timekeepers, circadian clocks. Circadian clocks allow organisms to coordinate behavior, physiology, and metabolism both internally and with their environment. The molecular mechanisms by which the circadian clock generates and sustains a 24-hour oscillation have been the focus of the field of circadian biology for the last three decades. Emerging from this effort has been the consensus that circadian clocks in all taxa share the common architecture of transcriptional/translational

feedback loops [1,2]. That is, circadian oscillations are generated by feedback loops in which the transcription and translation of one clock component induces the transcription and translation of a second component that feeds back to repress the first.

Rhythmic transcription and translation confer rhythmic accumulation of clock components, but it stands to reason that these clock components must also be inactivated and degraded for the cycle to proceed. Many examples are now known in which rhythmic post-translational modification, including ubiquitylation, leads to rhythmic degradation of clock components [3]. In this issue of *Current Biology*, however, van Ooijen *et al.* [4] establish that rhythmically controlled protein degradation is necessary to drive a circadian oscillation in transcriptionally competent cells of the picoeukaryote *Ostreococcus tauri*!

O. tauri, a unicellular marine alga, is the smallest known eukaryote [5] and expresses robust circadian rhythms. Introduction of transgenes consisting of *promoter:luciferase* transcriptional fusions in which the firefly luciferase gene is placed under the control of a clock-regulated *O. tauri* promoter allow the sensitive and non-invasive measurement of rhythmic gene transcription. Similarly, *promoter:coding-sequence:luciferase* translational fusions in which the clock-regulated promoter drives expression of a clock-regulated protein fused to luciferase allow the sensitive and non-invasive measurement of rhythmic protein accumulation and degradation [6]. The *O. tauri* genome is considerably reduced and this simplification extends to its clock, which apparently consists of a single feedback loop of the *O. tauri* orthologs of *Arabidopsis thaliana CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) and *TIMING OF CAB EXPRESSION 1* (*TOC1*) (Figure 1). This contrasts with the clocks of higher plants, in which multiple interlocked feedback loops are composed of components that are frequently redundantly specified — *A. thaliana CCA1* has a close and partially redundant relative, *LHY*, and *TOC1* is a member of a five-gene family of *PSEUDO-RESPONSE REGULATOR*s, each of which functions in the clock [7]. Although it had been argued that a clock of multiple interlocked feedback loops is necessary for flexible response to the
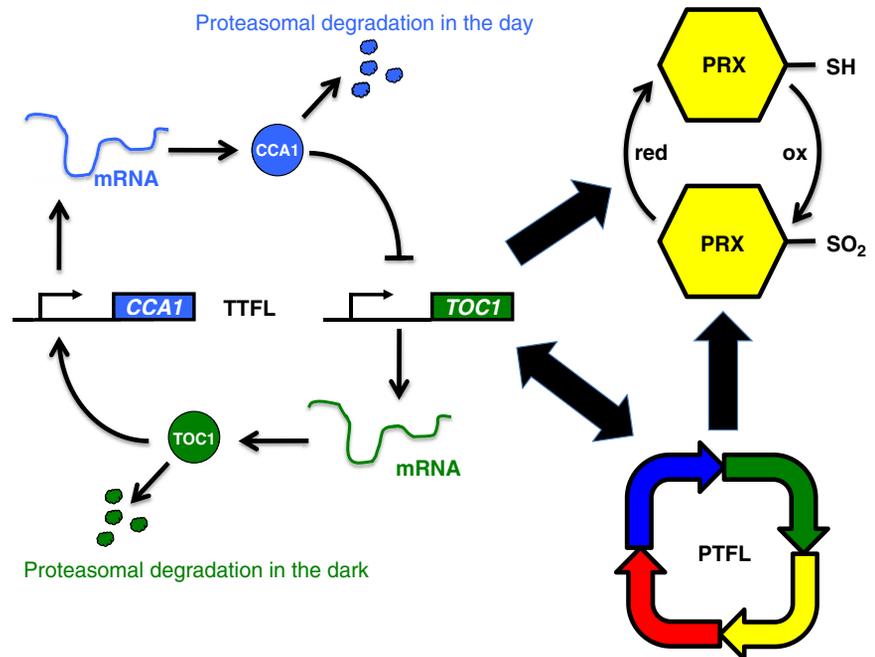

Figure 1. Two circadian clocks, one dependent on and one independent of transcription, in *Ostreococcus tauri*.

The *O. tauri* transcription/translation feedback loop (TTFL) requires transcription and translation of TOC1, which activates transcription of *CCA1*. CCA1 protein is a repressor of *TOC1* expression. Circadian-regulated proteasomal degradation of CCA1 is maximal in the day and diurnally-regulated proteasomal degradation of TOC1 peaks in the dark. Degradation is necessary for the maintenance of rhythmicity when cells are transcriptionally competent. This TTFL drives a circadian rhythm in the redox state of peroxiredoxin (PRX). However, in the absence of the TTFL (for example, in the dark, when transcription ceases), the PRX rhythm persists, driven by a largely uncharacterized post-translational feedback loop (PTFL). Normally the TTFL and PTFL are coupled.

environment [8], this single loop clock of *O. tauri* is nonetheless robust and flexible, exhibiting considerable complexity in its response to changing light conditions [9]. This makes *O. tauri* a very attractive system for the study of circadian clocks, especially for computational modeling of circadian networks.

van Ooijen *et al.* [4] measure degradation rates of translational fusions of CCA1 and TOC1 to firefly luciferase (LUC), each driven from their endogenous promoter. The degradation rate of CCA1–LUC is circadian regulated, peaking about 8 hours after subjective dawn, but that of TOC1–LUC is not. However, the degradation rate of TOC1–LUC varies diurnally, increasing in the dark. Pharmacological experiments demonstrate that the proteasome is necessary for degradation of both CCA1 and TOC1. Cells stop normal oscillatory behavior during treatment with proteasomal inhibitors, but upon wash-out of the inhibitor rhythmicity resumes with a delay corresponding to

the duration of treatment, suggesting that the circadian clock had paused. Scanning the circadian cycle with pulses of proteasomal inhibitor shows that clock function is sensitive to proteasomal inhibition at all times of day. This contrasts sharply to the phase-dependent effects of inhibition of either transcription or translation [10] and indicates that proteasomal degradation is critical for circadian timekeeping throughout the circadian cycle.

Transcription ceases when *O. tauri* is placed in prolonged darkness, but, surprisingly, circadian timing does not [10]. *O. tauri* displays a second type of rhythm in the redox state of peroxiredoxin (PRX) [10] (Figure 1), a member of a highly conserved and widely distributed family of anti-oxidant proteins [11]. Scavenging reactive oxygen species oxidizes the redox-reactive cysteine of PRX to sulphonic acid (sulphonylation) [11]. The circadian rhythm in PRX sulphonylation persists in constant light and in the presence of

pharmacological inhibitors of transcription and translation during constant light [10]. However, circadian rhythm in PRX sulphonylation in constant light is blocked by inhibition of the proteasome, establishing the necessity of proteasomal function to rhythmicity [4]. In the dark, however, transcription ceases. Under these conditions, proteasomal inhibition failed to block rhythmic PRX sulphonylation, indicating that proteasomal degradation was necessary for rhythmicity only under conditions in which protein synthesis persisted. However, inhibitors of other post-translational modifications had similar effects on the period of PRX sulphonylation as they did in the light [9]. This argues that the transcription/translation feedback loop (TTFL) and the post-translational feedback loop (PTFL) are normally tightly coupled under physiologically relevant conditions. However, in the abnormal and stressful condition of extended dark, encountered perhaps when *O. tauri* cells are carried deeply into the water column, the transcription/translation feedback loop is absent due to the cessation in transcription. The cessation of transcription is presumably a survival mechanism to endure a period of energy starvation. Nonetheless, the persistence of the post-translational rhythm in PRX sulfonylation suggest that there is still a survival value associated with rhythmicity, presumably in coordinating metabolism in these near-dormant conditions [4].

PRX proteins are widely distributed among taxa. Apparently rhythms in PRX sulphonylation are similarly widespread, because PRX proteins exhibit a robust circadian rhythm in PRX sulphonylation in human red blood cells [12]. This is a striking result, because human red blood cells lack nuclei and so are incapable of transcription. Of course, the demonstration that the cyanobacterial KaiA, KaiB, and KaiC proteins together with ATP are sufficient to reconstitute a robust temperature-compensated *in vitro* rhythm in KaiC phosphorylation had already established that rhythmicity was possible without transcription and translation [13], but now this has been extended to two eukaryotes of quite distinct lineages. Interestingly, 50 years ago it was observed that circadian rhythms in photosynthesis persist in enucleated

Acetabularia major and *A. crenulata* [14] and almost 40 years ago a rhythm in respiration was reported in dormant onion seeds [15]. Obviously, these multiple observations of circadian rhythmicity without *de novo* transcription in cyanobacteria, *Acetabularia*, *O. tauri*, onions, and humans fully refute the general necessity of transcription for circadian clock function.

Are there two circadian clocks present in most cells, one based on transcription/translation feedback loops and one based on transcription/translation-independent mechanisms (Figure 1)? Certainly there are multiple examples of circadian rhythmicity in genotypes in which the known transcription/translation feedback loop mechanism is disrupted [16]. Although it seems premature to claim the ubiquity of these two types of clocks, it nonetheless seems likely that the exploration of the interaction between these two clock mechanisms is likely to offer important insights. The implications of these two types of clocks for the evolution of circadian rhythmicity are profound.

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Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA.
E-mail: C.Robertson.McClung@Dartmouth.Edu

Synaptic Growth: Dancing with Adducin

Manipulations of the actin-capping protein adducin in *Drosophila* and mammalian neurons provide new insights into the mechanisms linking structural changes to synaptic plasticity and learning. Adducin regulates synaptic remodeling, providing a molecular switch that controls synaptic growth versus disassembly during plasticity.

Robin J. Stevens and J. Troy Littleton

Developing neural circuits are often highly plastic and not only form new synaptic contacts, but also eliminate unnecessary or redundant synapses. Once the brain has matured, extensive remodeling of circuits is rare, but connections between neurons can be

modified in an activity-dependent fashion as well as in response to injury or disease [1]. Alterations of synaptic connections are hypothesized to underlie learning and memory and can occur through several mechanisms. The strength of a synapse can be increased or decreased by changing the properties of presynaptic release or