

The circadian clock coordinates metabolic activity in animals, plants, and certain microorganisms. Recent studies on circadian rhythms, discussed in this issue's Molecular Biology Select, provide evidence that dietary fat and a nuclear receptor ligand have the capacity to regulate circadian gene expression and daily rhythms of animal behavior. Other reports explore the impact of circadian rhythms on freezing tolerance in plants, DNA supercoiling in cyanobacteria, and the timing of spawning in coral.

Up All Night, Eat All Day

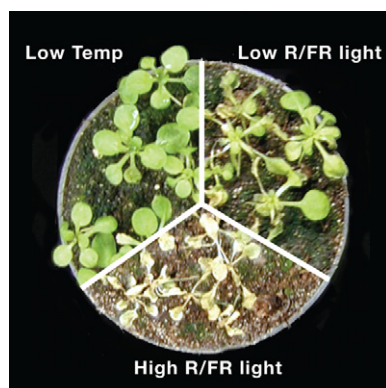
Mice with a disrupted circadian clock due to loss of the *Clock* gene eat excessively and grow obese, providing clear evidence that the circadian cycle can regulate metabolism. However, it has been unclear whether the converse is also true—that is, do changes in metabolism alter circadian rhythms? According to Kohsaka et al. (2007), the answer is yes. They show that a high-fat diet changes multiple aspects of circadian physiology and behavior in mice. When put in constant darkness, mice fed high-fat food had a circadian period that was longer than that of mice on normal chow; the effect was observed in the first 2 weeks of the experiment, before differences in body weight become apparent. Diet also affected diurnal patterns of behavior. Compared to mice on a normal diet, mice fed a high-fat diet ate less food during the night (the normal feeding time for mice) and more during the day. These changes were matched at the molecular level, with mice on a high-fat diet exhibiting diminished amplitudes of clock gene expression, with the greatest decrease observed in adipose tissue. High-fat food also altered the circadian pattern of expression of numerous other factors, including leptin, insulin, neuropeptides, and among nuclear receptors previously implicated in regulating circadian clock genes. This work shows that the effects of a high-fat diet are not just limited to obesity and its complications, a notion that has clear relevance for assessing the full impact of diet on human health. A. Kohsaka et al. (2007). *Cell Metab.* **6**, 414–421.

Heme Has a Rev-erberating Impact on Circadian Genes

The transcription factor Bmal1 is a master regulator of the mammalian circadian clock that controls the expression of numerous clock-related genes, including the *Period* and *cryptochrome* genes. Previous work has established that the circadian pattern of Bmal1 expression is itself regulated by the orphan nuclear receptor Rev-erb α . Now Raghuram et al. (2007) and Yin et al. (2007) identify heme (a prosthetic group consisting of an iron atom bound by a porphyrin ring) as the ligand for human Rev-erb α , and show that heme regulates expression of Rev-erb α target genes, including *Bmal1*. Heme is an essential component of many proteins, and its intracellular concentration has been shown to vary in a circadian manner. The two groups provide evidence that Rev-erb α is regulated by binding of heme. A mutant Rev-erb α that is unable to bind heme had a diminished capacity to recruit the nuclear receptor corepressor (NCoR)-histone deacetylase 3 (HDAC3) complex to Rev-erb α target genes. Also, heme enhances the interaction between Rev-erb α and NCoR-HDAC3, and this pathway regulates not only BMAL1 but also glucose 6-phosphatase, whose action is required for glucose output from liver cells. Hence, these findings provide a provocative mechanistic link connecting metabolism and circadian rhythm. Future work may establish whether physiological variation in heme concentration regulates Rev-erb α activity in vivo and, if so, in what tissues and process it is most critical for coordinating aspects of the circadian clock and energy metabolism.

L. Yin et al. (2007). *Science*. Published online November 15, 2007. 10.1126/science.1150179.

S. Raghuram et al. (2007). *Nat. Struct. Mol. Biol.* Published online November 25, 2007. 10.1038/nsmb1344.



Survival of *Arabidopsis* plants following a single freeze/thaw cycle with 4 days prior acclimation to either low temperature, high red/far-red (R/FR) light or low R/FR light. Image courtesy of K. Franklin.

The Long Twilight Struggle of the Plant Cold War

To prepare for the challenges of winter, many plants take advantage of exposure to cool temperatures (above but near 0°C) to acquire what is known as freezing tolerance. A new report by Franklin and Whitelam (2007) indicates that plants also take heed of another environmental signal, a reduction in light quality, to initiate the program of freezing tolerance. Light quality is distinct from light intensity and is measured by the ratio of red to far-red (R/FR) light; the ratio is highest in direct sunlight and lower in the shade of vegetation or in twilight, which is prolonged at higher latitudes. Previous work in the model plant *Arabidopsis thaliana* has shown that freezing tolerance is mediated by the CBF family of transcriptional activators and their downstream targets, including the *COLD REGULATED (COR)* genes. In their current study, the authors now show that prolonged exposure to low R/FR light can induce expression of *COR* genes at ambient temperatures (16°C), triggering freezing tolerance. To establish the signaling pathways mediating this effect, they examined the ability of photoreceptor proteins called phytochromes to regulate *COR* gene expression. They show that loss of either phytochrome B or D enhances the expression of *COR15a* and that loss of

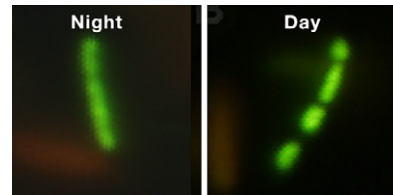
phytochrome D enhances freezing tolerance independent of light quality. The authors also show that expression of *CBF* genes is tightly linked to the circadian clock and is markedly enhanced in low R/FR light. These findings demonstrate how a plant can integrate different environmental signals, in this case light quality and temperature that converge at the level of *CBF* expression and stability, to best prepare for potential changes in its environment. Future efforts may examine whether seasonal changes in day length in addition to the changes in light quality also impact *COR* gene expression at ambient temperatures.

K.A. Franklin and G.C. Whitelam (2007). *Nat. Genet.* **39**, 1410–1413.

DNA in Cyanobacteria Relaxes at Night

Prokaryotic cyanobacteria display circadian rhythms that enhance their fitness and may reflect their dependence on the sun for obtaining energy via photosynthesis. To gain insight into the mechanisms contributing to circadian regulation of gene expression in cyanobacteria, Woelfle et al. (2007) have studied the behavior of a nonessential plasmid introduced into the cyanobacterium, *Synechococcus elongatus*. They observed that the plasmid DNA was more highly supercoiled early in the day and became more relaxed during the night. As evidence that this might contribute to the global regulation of gene expression, cyanobacterial promoters on the introduced plasmids were able to drive expression of a luciferase reporter gene in a circadian fashion. The authors show that the daily change in DNA topology is dependent on KaiC, a core component of the KaiABC circadian oscillator in cyanobacteria; however, unlike the KaiABC system, the circadian change in plasmid DNA topology is not maintained under conditions of constant darkness. This finding suggests that there is also a light-dependent signal that couples DNA topology to circadian regulation by KaiABC. Future work may also connect the dots between KaiC-containing protein complexes and circadian-driven changes in DNA topology.

M.A. Woelfle (2007). *Proc. Natl. Acad. Sci. USA* **104**, 18,819–18,824.



In individual cyanobacterial cells, DNA (green) is relaxed at night and compacted in daytime. Image courtesy of C. Johnson.

Coral “CRY” for the Moon



Colonies of the coral *Acropora millepora* synchronously release bundles of eggs and sperm during mass spawning events. Photo courtesy of R. Reef.

In recent work, Levy et al. (2007) plumb the depths of the ocean to gain insight into the evolution of circadian behavior in simple animals. They discovered that coral, a simple multicellular organism near the base of the animal tree, expresses cryptochrome (*cry*) genes, which encode light-sensitive flavoproteins. Studying the coral *Acropora millepora*, the authors show that the expression of both *cry1* and *cry2* oscillates in a circadian fashion when coral are exposed to normal light-dark cycles, but not when they are maintained under conditions of sustained darkness. This suggests that CRY proteins in coral are involved in clock entrainment, similar to what has been observed for CRY proteins in the fruit fly *Drosophila*. The discovery of these genes provides insight into how corals display photosensitive behavior despite having no specialized organs dedicated to light detection. Their work may also reveal mechanisms that control mass spawning, a remarkable and puzzling event of the coral lifecycle. Colonies of coral release gametes synchronously, but only on a few nights each spring and always a few days after a full moon. In an experimental colony of *A. millepora* located in Australia's Great Barrier Reef, the authors observed that expression of *cry2*, but not *cry1*, is markedly higher on nights with a full moon compared to nights

with a new moon, suggesting that CRY2 may be one factor dictating the timing of spawning. Future work may explore why expression of *cry2* and not *cry1* is affected by moonlight.

O. Levy et al. (2007). *Science* **318**, 467–470.

Robert P. Kruger