# Bacterial circadian clocks set by metabolism, not light

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An image of Cyanobacteria, Tolypothrix. Credit: Wikipedia / CC BY-SA 3.0

Most organisms on Earth, from bacteria to humans, possesses a circadian clock—a biological mechanism that synchronizes activities such as rest or growth to daily changes in a 24-hour day. Although commonly thought to be tied to the day-night cycle, the circadian clock actually appears to be set by metabolic rhythms, according to a new study by scientists from the University of Chicago. By genetically reengineering cyanobacteria to be able to feed on sugar, the team discovered that the cyanobacterial clock was unaffected by light or dark, but responded only to metabolism.

The findings, published in *Cell Reports* on Dec. 10, 2015, offer a glimpse into the evolutionary roots of the circadian clock and point to potential applications in synthetic biology.

"The question we are asking is how the circadian clock learns what time of day it is," said senior study author Michael Rust, PhD, assistant professor of molecular genetics and cell biology. "In cyanobacteria, that answer seems to be especially simple—the clock proteins sense the metabolic activity in the cell."

The circadian rhythm allows organisms to anticipate the regular environmental changes that occur during a 24-hour day and synchronize their biology accordingly. For humans, this manifests as preferred periods of time for sleep, eating or activity, which are linked to the day-night cycle. Cyanobacteria, also known as blue-green algae, follow a similar pattern, engaging in photosynthesis and growth during day and "rest" and energy conservation at night.

The biological mechanism that drives the circadian rhythm is known as the circadian clock. Cyanobacteria possess one of the simplest known circadian clocks—the KaiABC system, a complex of proteins that undergoes a 24-hour biochemical cycle, which triggers oscillations in gene expression and physiological activity. This clock can be entrained, or "set," when the bacteria are exposed to different periods of light and dark, similar to how human internal clocks adjust after long-distance travel.

Despite this link, studies have found no evidence that the Kai system receives any information about light exposure. Instead, Kai proteins are sensitive to metabolic signals.

To study the precise relationship between the circadian clock, light and metabolism, Rust and his colleagues created genetically engineered cyanobacteria that were able to feed on sugar in addition to normal photosynthesis. As these bacteria are able to survive in complete darkness just as well as light, the team could separately test the effects of metabolism and light.

They began by comparing normal cyanobacteria to genetically altered ones. Under constant light exposure, both normal and altered cyanobacteria will maintain a regular circadian rhythm. Introducing periods of darkness easily resets the clocks of normal cyanobacteria.

However, darkness had no effect on the clocks of altered cyanobacteria, which had access to plentiful sugar. The team discovered that ATP and ADP—two molecules that are central to metabolism and are known to modulate the Kai system—were maintained in a steady ratio during darkness, suggesting that sugar feeding was responsible for stabilizing the clock.

"We found that the bacterial circadian clock responds only to metabolic activity," Rust said. "If growth and metabolism are supported, the clock doesn't seem to care whether it's light or dark."

The team then tested the effects of sugar availability. They observed a regular circadian rhythm when they kept altered cyanobacteria in constant darkness, with a constant supply of sugar. However, when they removed sugar for periods of time, simulating a period of "nighttime" starvation, the bacterial circadian clocks would quickly reset. Different batches of cyanobacteria, on different feeding schedules, synchronized their clocks according to when they access to sugar.

"By looking at the molecular state of their clock, we found the bacteria tracked when we were feeding them and set their internal times to that schedule. They basically "learned" when the sugar was coming," Rust said.

Cyanobacteria are one of the most ancient forms of life on Earth, and coupled with the universal nature of the circadian clock, this study indicates that its fundamental function is to help schedule different metabolic activities. Many microbes, such as human gut bacteria and some disease-causing bacteria, have genes similar to the cyanobacterial clock genes. But it is still unclear if they have a circadian rhythm. A better understanding of their circadian clocks and metabolism could possibly inform research efforts to promote healthy gut bacteria or neutralize harmful ones, Rust suggests.

The discovery that the circadian clock is tied to metabolism and not light also raises numerous possibilities in synthetic biology. In previous experiments, Rust has demonstrated that Kai proteins isolated in test tubes are able to maintain their ability to function as a biological clock and respond to metabolic signals. Because its time-keeping function is independent of photosynthesis, efforts to install the Kai system into other microbes to carry out scheduled tasks become more promising. As for what better knowledge of circadian clocks could unlock in the distant future, Rust entertains a tantalizing possibility.

"In the historical development of electrical circuits, engineers found that synchronizing each step of a computation to an internal clock made increasingly complicated tasks possible, ultimately leading to the computers we all use today," he said. "Perhaps in the future we'll be able to use synthetic clocks in engineered microbes in a similar way."

**More information:** Controlling the Cyanobacterial Clock by Synthetically Rewiring Metabolism, *Cell Reports*, 2015.

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