



Circadian clock advances



Bioclock Fitness

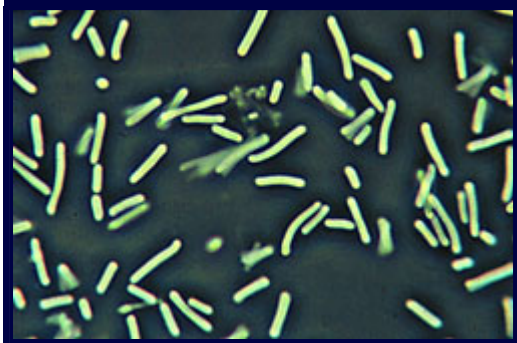
A traveler experiences jet lag when his or her internal clock becomes out-of-synch with the environment. Seasonal Affective Disorder, some types of depression, sleep disorders and problems adjusting to changes in work cycles all can occur when an individual's biological clocks act up. Recent studies have even found links between these molecular time-pieces and cancer.

Microscopic pacemakers—also known as circadian clocks—are found in everything from pond scum to human beings and appear to help organize a dizzying array of biochemical processes. Despite the important role that they play, scientists are just beginning to understand the benefits that these internal pacemakers provide when they work and the problems they cause when they malfunction.

A study¹ performed by researchers at Vanderbilt University and published in the Aug. 24 issue of the journal *Current Biology* sheds new light on this issue. Using blue-green algae—the simplest organism known to possess these mechanisms—the researchers report that the benefits of biological clocks are directly linked to environments with regular day/night cycle and totally disappear in conditions of constant illumination.

¹ "The adaptive value of circadian clocks; an experimental assessment in cyanobacteria" (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15324665)

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Courtesy of Carl Johnson

Photomicrograph of the *Synechococcus* strain of blue-green algae used in the experiment

“Circadian clocks are so widespread that we think they must enhance the fitness of organisms by improving their ability to adapt to environmental influences, specifically daily changes in light, temperature and humidity,” says Carl H. Johnson, professor of biological sciences and Kennedy Center investigator who directed the study. “Some people have even suggested that, once invented, these clocks are such a powerful organizational tool that their benefits go beyond responding to external cycles. However, there have been practically no rigorous tests of either proposition.”

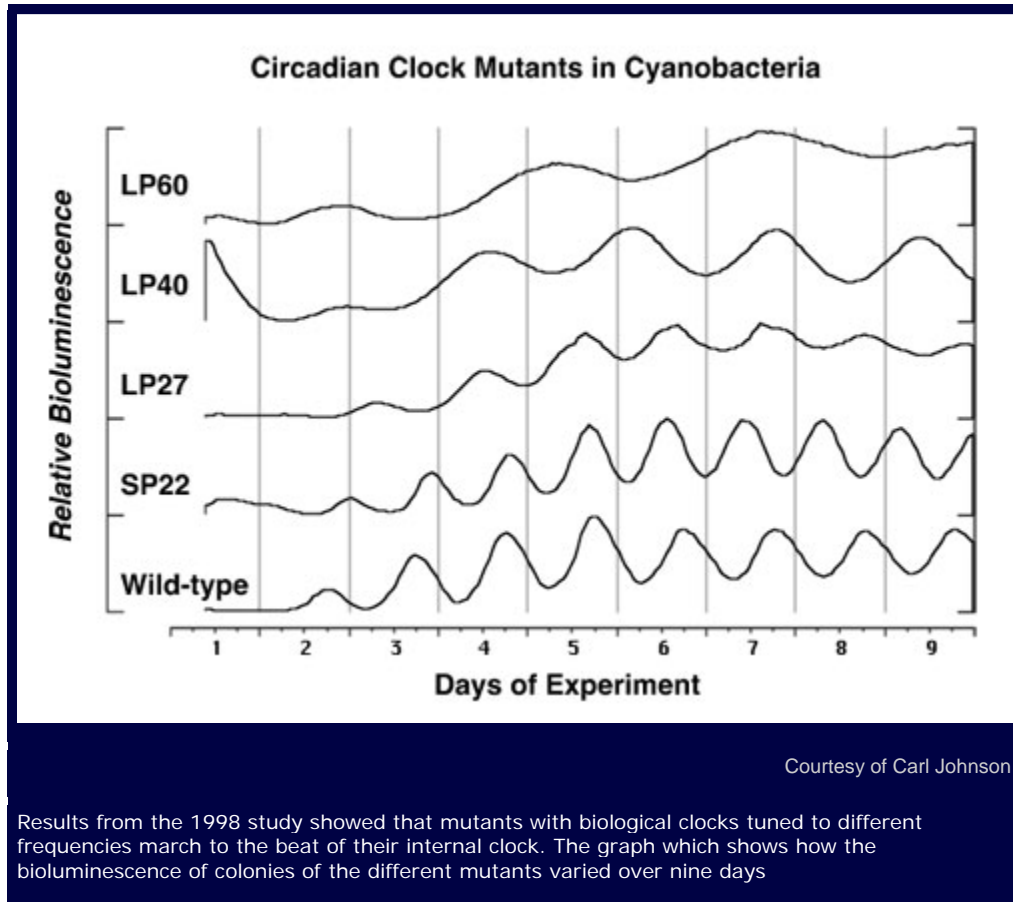
To test these ideas directly, Johnson's research team used genetic engineering techniques to completely disrupt the biological clocks in one group of algae and to damp the frequency of the

clocks in a second group. The researchers were careful to employ “point” mutations in the clock genes that didn't stunt the growth of the microscopic plants.

They then mixed the algae with disrupted clocks with algae with normally functioning clocks. When the mixture was placed in an environment with a 24-hour day/night cycle, the normal algae grew dramatically faster than those that lacked functional internal timers. The normal algae also outperformed the algae with the damped clocks, but by a smaller margin.

The result was presaged by a series of experiments that Johnson conducted in 1998 with Susan S. Golden from Texas A&M University and Takao Kondo from Nagoya University . In the previous experiments, the researchers created two new algae strains with clocks of 22 hours and 30 hours. (The frequency of the biological clocks in normal blue-green algae is 25 hours.) They created mixed colonies by combining the strains in pairs: wild type and 22 hour; wild type and 30 hour; 22 hour and 30 hour. Then they put these mixed cultures into incubators with three different light-dark cycles—22 hours, 24 hours and 30 hours—and monitored them for about a month.

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When they pulled the cultures out, the researchers found that the strain whose internal clock most closely matched the light-dark cycle invariably outgrew the competing strain. In fact, they found that the selective advantage of having the correctly tuned biological clock was surprisingly strong: The strains with matching frequencies grew 20 to 30 percent faster than the out-of-synch strains.

The second part of the current experiment was designed to test whether the biological clocks also provide an intrinsic advantage, a hypothesis advanced by the late Colin Pittendrigh of Stanford. He suggested that circadian clocks might be beneficial even in an unchanging environment. There was some indirect support for this proposition. In one experiment, for example, populations of the fruit fly, *Drosophila melanogaster*, were raised in constant illumination for hundreds of generations. Nevertheless, their biological clocks continued to function, suggesting that they continue to have adaptive value.

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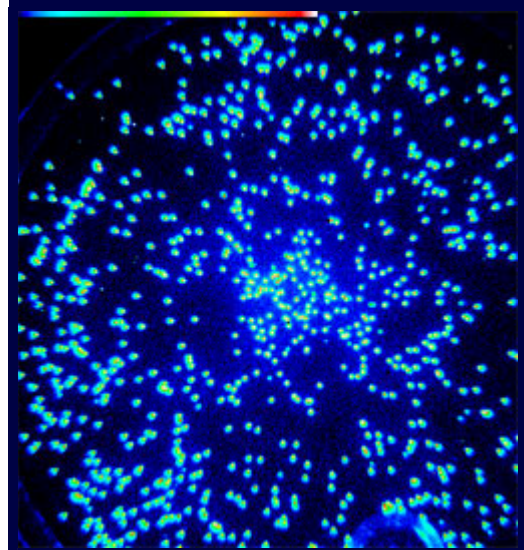
When the algae strains were placed in a chamber with constant light, however, the researchers were surprised to discover that the shoe was on the other foot: The algae with the disrupted internal clock divided and grew at a slightly faster rate than their clockwatching cousins, both those with natural biological clocks and those whose clocks were damped.

“This was the most surprising result of our study,” says Johnson. “Under constant conditions, the circadian clock system is of no benefit and, in fact, might even be bad for the algae.”

The scientist doesn't know for certain why this happens, but he has some ideas. The microscopic plants use their biological clocks to turn their photosynthesis system on and off. In a normal 14-hour day/night cycle, this allows the microscopic plant to maximize the amount of chemical energy it can extract during daylight.

“In constant illumination, however, the biological clocks may keep shutting down photosynthesis in expectation of the darkness that never comes,” says Johnson.

Co-authors on the study are post-doctoral fellows Mark A. Woelfle and Yan Ouyang and graduate student Kittiporn Phanvijhitsiri. The research was supported by the National Institutes of Health.



Courtesy of Carl Johnson

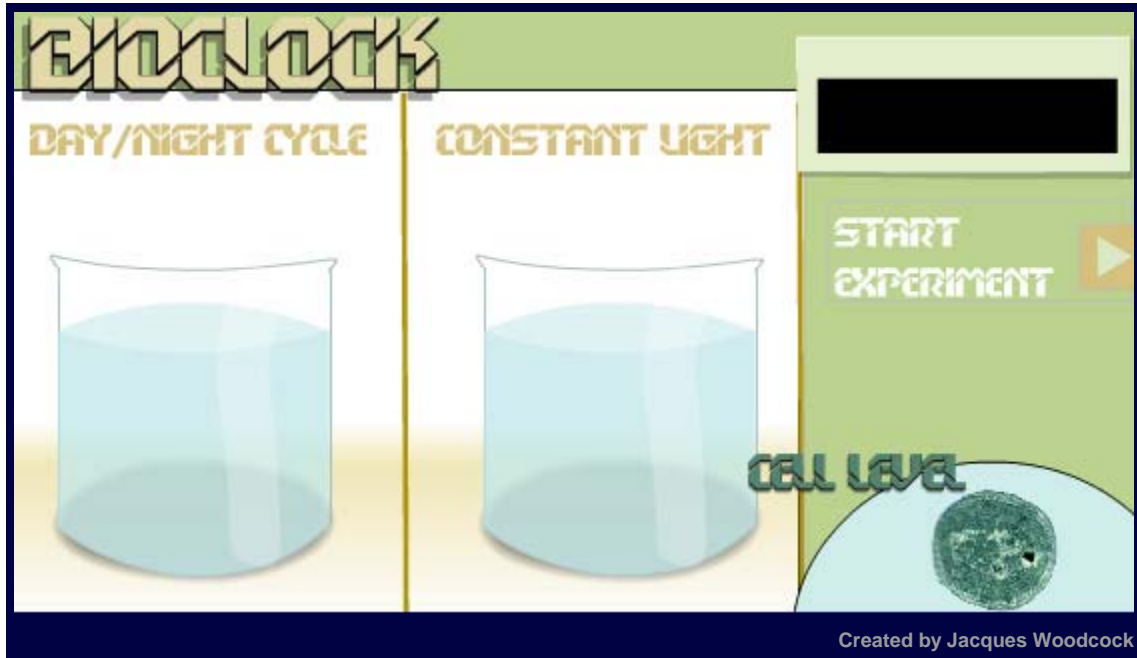
In order to study the biological clocks in blue-green algae, researchers have inserted a gene for the fluorescent protein luciferase into the algae's DNA at a position where its production is controlled by the bioclock

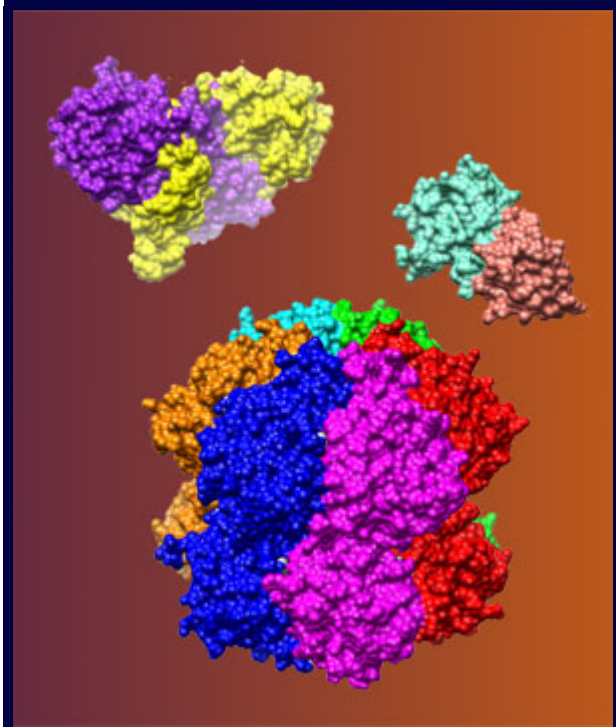


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Fitness Animation





Created by Sabuj Pattanayek

Illustration of the 3-D structure of the three blue-green algae clock proteins. KaiA subunits are colored yellow and purple; those of KaiB are colored aquamarine and salmon; and KaiC subunits are colored red, green, aquamarine, gold, blue and violet. The structure of KaiA was determined by researchers at Texas A&M; that of KaiB was done by a group of researchers at the University of Toronto; and the structure of KaiC was established by the Vanderbilt researchers.

3-D structure of biological clockwork revealed

In the last six months, scientists have taken an important step towards deciphering the inner workings of circadian clocks, an understanding that could ultimately lead to novel treatments for clock-related disorders, such as jet lag, sleep disorders and some types of depression. They have determined the three-dimensional structures of the three proteins that make up the simplest known biological clock, the one that operates in blue-green algae.

The structures of the two smaller biological clock molecules were determined by groups at Texas A&M, Nagoya University and the University of Toronto. Now two Vanderbilt researchers—Martin Egli, associate professor of biochemistry, and Carl H. Johnson, professor of biological sciences—report that they have solved the structure of the third and largest of these biological clock proteins.

Blue-green algae are the simplest organism known to have biological clocks. As in higher organisms, like human beings, the clocks in these single-celled plants regulate gene expression, cyclically turning genes on and off. Three proteins—KaiA, KaiB, and KaiC, named after the Japanese word for cycle—are the key components of the algae's clock; without any one of them, the clock does not work.

Egli and Johnson published the crystal structure—a kind of molecular "snapshot"—of KaiC in *Molecular Cell*² in August and reported additional features of the protein in the Sept. 21 issue of the *Proceedings of the National Academy of Sciences*.³

Though the proteins that make up the gears and springs of the circadian clock in these simple plants differ from those that form the human clock, it is quite likely that the fundamental biochemistry of clock function has remained unchanged, the investigators say.

² "Visualizing a Circadian Clock Protein: Crystal Structure of KaiC and Functional Insights;" *Molecular Cell*; 13 August 2004 ;"
(<http://www.molecule.org/content/article/abstract?uid=PIIS1097276504004356&highlight=egli&highlight=circadian>)

³ *Proceedings of the National Academies of Sciences*; 21 September 2004
(<http://www.pnas.org/cgi/content/abstract/101/38/13933>)

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"Hopefully, some of the basic principles that we uncover at the biochemical level [in blue-green algae] will guide the research in the mammalian systems," Johnson says.

With the structures of KaiA, KaiB and KaiC published in the last few months, the field is in a position to tackle complex questions of clock function, Egli says. "There's been this culmination of five years worth of work, all in a matter of months. It's a really exciting time."

The KaiC structure is already providing hints to its biochemical operations, but the investigators stress that the work is still in an early stage.

"Even though we've learned things from the structure," Egli says, "the big question still is: what are the underlying biochemical mechanisms that allow organisms to control their rhythms so precisely?"

Six KaiC molecules appear to group together to form a ring-like structure that looks something like a mechanical gear—oddly appropriate, given its function as the core of the timepiece. KaiA and KaiB associate with the KaiC ring depending on a biochemical reaction called phosphorylation. Egli and Johnson's work has identified three phosphorylation sites on KaiC; mutation of any of these sites turns off the clock.



Photo by Neil Brake

Johnson inspecting a beaker of blue-green algae.

The KaiC structure reveals unexpected evolutionary relationships to proteins that manufacture the energy molecule ATP and to DNA pumps. What these similarities mean is still anyone's best guess, Egli says, adding "I think there must be some unusual mechanism."

In addition to opening new avenues for the treatment of some sleep disorders and forms of depression, clock research raises questions about timing of medication dosing. There may be optimum times of day for hitting a particular target, depending on the cycling of genes on and off. Other groups are investigating whether the timing of chemotherapy, for example, can reduce side effects and enhance efficacy, Egli says.

According to the researchers, circadian clocks are increasingly being recognized as fundamental to biology.

"The emerging idea is that the organism is basically a clock shop—that everything is oscillating," Johnson says. "One function of the brain, particularly certain parts of the brain, is to keep all of that organized and synchronized. The brain acts as a pacemaker for all of the other clocks in all of the other cells in the body, even in your big toe."

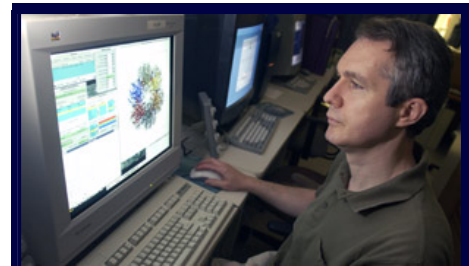


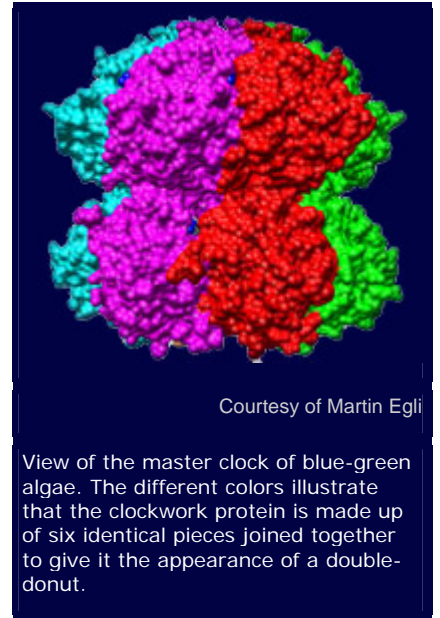
Photo by Neil Brake

Martin Egli viewing image of clock protein

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The research was supported by the National Institutes of Health, the National Science Foundation and a Vanderbilt University Medical Center Intramural Discovery Grant.

-- Leigh MacMillan



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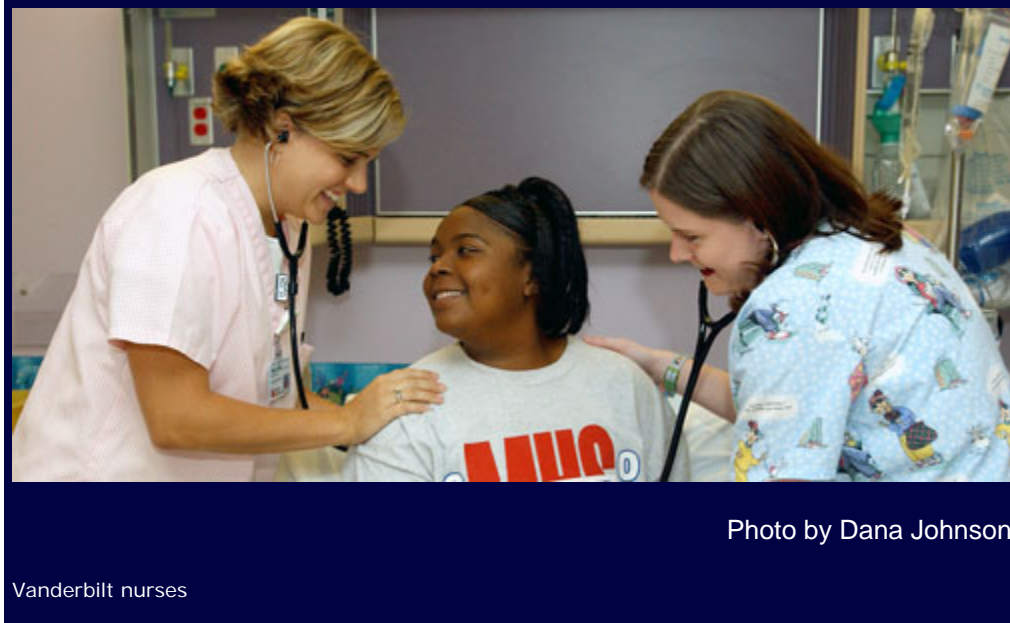


Photo by Dana Johnson

Vanderbilt nurses

Nurses studied in new phase of circadian clock research

Could circadian clock genes play a key role in determining what time shift best suits each individual nurse? A team of Vanderbilt researchers thinks so, and they hope to prove that theory in a new study involving nurses at Vanderbilt's Medical Center.

Professors Carl H. Johnson and Douglas McMahon from biological sciences have teamed up with Nancy Wells, director of nursing research and research professor of nursing, and a number of nursing students to test the information they've gathered on clock genes using the largest population of shift workers at Vanderbilt- the nursing staff. "We know from lower organism research that genes determine your biological clock," says Johnson. "That's very likely to be the case in humans, but at this stage in the research it is not known," he adds.

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Nancy Wells

The team of researchers hopes to determine who is going to have trouble adjusting to shift work based on their biological or circadian clock genes. "The knowledge we gain will be in learning what genes may be related to poor shift work in the rhythm of nurses," says Wells. "In the long run, the more we know about how people vary in terms of ability to shift rhythms, the better able we will be to help them either make job choices that fit with their genetics, so to speak, or learn strategies that will help them go against the grain," she says.

Johnson says clock genes have been shown to be closely related to problems like insomnia, certain cancers, as well as the way a person responds to certain medications. Down the road, he says research like this could lead to tailor-made therapies that compensate for those genetic predispositions. "This may allow you to design therapies based on genetics that might be targeted therapy for people who have trouble with treatments, cancer treatments, sleep disorders, jet lag," says Johnson.

Participants will take a 10-minute survey about their sleep-wake habits, how they adapt to shift work, and other related questions, and have their blood drawn. Johnson says the blood samples will be used to look for different

forms of clock genes that might affect whether a nurse has a fast or slow clock that might alter sleeping and waking patterns. The researchers hope to sign up 400 nurses.

-- Heather L. Hall

