

Around the Clock



by Sarah C.P. Williams

illustration by Michael Kirkham

[Download PDF
of this Story](#)

In nearly every place on Earth, life pulses with a daily rhythm. As the sun rises over the savannah, plants spread their leaves and animals blink open their eyes. As light hits the oceans, night-loving crabs bury themselves in sand and fish emerge from deep water in search of food. In towns and cities, people wake to alarm clocks and read the morning news. These routines of life are more than a matter of habit and convenience. They are driven by deeply rooted biological programs that keep the planet's inhabitants on a 24-hour schedule called a circadian rhythm.

The circadian clock influences far more than daily habits, according to a few decades of research. Obesity, diabetes, cardiovascular disease, liver disease, cancer, and depression have all been linked to malfunctions of the internal timekeeping scheme. Almost every cell in a living organism, it turns out, sees daily fluctuations in levels of genes and proteins, and when these fluctuations are dampened or stopped, things can go awry.

"The circadian system has its tentacles around everything," says HHMI Investigator Michael Rosbash. "It's ticking away in almost every tissue in the human body." And in plants, too—including major food crops—circadian rhythms are tied to disease susceptibility, growth rate, and fruit size.

Studies by Rosbash and other HHMI scientists are revealing links between the internal clock and health, and they are detailing the complex cellular machinery that drives the circadian clock in organisms from plants to flies to humans. They hope that discovering these components—like understanding the gears of a wristwatch or the swinging pendulum of a grandfather clock—will allow them to fix the system when it's off-kilter, or at least prevent it from losing track of time when a person travels across time zones or pulls an all-nighter.

Noon

The sun hangs high in the sky and shadows have all but disappeared. At Sonny Bryan's Smokehouse in Dallas, the line of lunchtime patrons snakes out the door and the smell of Texas barbecue wafts across the parking lot. But it's not just the smell that has everyone's stomachs growling. Inside each waiting customer's body, the digestive system is preparing for the midday meal. Anticipating food, hormones in the liver and stomach spike, and neurons in the brain send hunger signals throughout the body.

Across the street, on the University of Texas Southwestern Medical Center campus, scientists in Joseph Takahashi's lab can't smell the barbecue, but they can tell that it's the middle of the day just by glancing at their cages of mice or by measuring protein levels in cells—both cycle in predictable ways each day.

Twenty years ago, Takahashi, an HHMI investigator, watched daily behavior patterns of mice to discover the first gene—dubbed Clock—that controls the circadian rhythm in mammals. He found that healthy mice follow a 24-hour schedule of sleeping, eating, and exercising, even in steady darkness. But without normal CLOCK proteins, mice kept in a dark environment alternate these behaviors at all times of the day and night. Since [that 1994 discovery](#), researchers have identified a host of other circadian control genes.

“This field has gone through many different phases,” says Takahashi. “At the turn of the century, it was incredibly exciting because all these genes were being discovered. Then, we found that clocks were in almost every cell of the body. Now, we’re beginning to understand the role of clocks in cell and organismal physiology and metabolism.”



Circadian cycles in mammalian cells are the focus of Joseph Takahashi's work. Photograph by Brandon Wade.

In a TEDx Talk, Joseph Takahashi explains the clock in our genes and reminds us to keep in synch.

Before Takahashi's work on mammalian clock genes, former HHMI Investigator Michael Young at Rockefeller University, along with Rosbash and his Brandeis University colleague Jeffrey Hall, had already cloned circadian genes from the fruit fly *Drosophila melanogaster*. They'd established that the genes' expression cycled over 24 hours using a feedback system: in the flies, genes called *timeless* and *period* produced proteins that paired up, traveled to cell nuclei, and turned off the very genes that encoded them. Interestingly, the mammalian CLOCK and BMAL1 proteins could regulate the fly *period* and *timeless* genes, and this led to the concurrent discovery of the fly versions of the Clock and Bmal1 genes by Takahashi and Steve Kay and by Rosbash and Hall. Young then went on to discover mutants that made this cycle last longer or shorter than the typical day length, and Rosbash and Hall pinpointed and cloned a host of other genes that influenced the clock.

Takahashi and others found that in mammals, similar cycles occur: In mammalian brain cells, the CLOCK protein and its partner BMAL1 pair up and turn on *Period* and *Cryptochrome* genes. Once the *Period* and *Cryptochrome* proteins reach a certain level, however, they shut down CLOCK and BMAL1. The competition between the two sets of proteins leads to an endlessly fluctuating cycle—one that takes 24 hours to complete. Levels of CLOCK and BMAL1 peak during each day, and *Period* and *Cryptochrome* rise each night. As they rise and fall, the quartet of proteins turns on and off many other genes throughout the body. That's how the circadian rhythm has such wide-ranging effects.

An overview of the components that make up the mammalian molecular clock.

Recently, Takahashi focused on how the circadian cycle influences the liver. “If you look in the liver at the targets of CLOCK and BMAL1, and plot those genes on a chart of metabolic pathways, essentially the entire chart would be covered,” he says. “Every fundamental metabolic pathway is under circadian control.”

The link between the clock and metabolism explains why humans and animals tend to eat at the same time every day, and why digestive molecules increase even before a person sits down for a meal. But that isn’t all—Takahashi’s group discovered that CLOCK and BMAL1 not only bind many of the metabolism genes, they also recruit RNA polymerase II—a protein needed to read every gene in the body—throughout the genome on a circadian basis.

The implications of this observation are huge, Takahashi says, and suggest that the majority of genes in the body may be under the control of the clock through the cycling of RNA polymerase II.

“What this means is that, even genes that we can’t directly measure as having circadian cycling are, in fact, cycling in some ways,” he says.

In *Drosophila*, Young has used high-throughput genetic technology to screen all 14,000 fly genes for daily cyclical activity. In the fly’s head alone, he found, the levels of more than 500 genes fluctuate in circadian patterns.

But over the past few years, scientists have also realized that the circadian rhythm doesn’t just control protein levels by altering when genes are transcribed from DNA to RNA, the first step in gene expression.

Rosbash, also still working in *Drosophila* as a model for circadian rhythms, is revealing the many ways that protein levels are controlled by the clock. He uses a technique called nascent RNA sequencing to get a snapshot—at any given time—of which genes in a cell are being actively transcribed into strands of so-called nascent RNA. This is different from looking at what strands of processed messenger RNA are present.



Michael Rosbash studies the daily changes that occur in the circadian neurons in the fruit fly brain. Photograph by Paul Feters.

“What we see is a disconnect between the nascent RNA profiles and some messenger RNA profiles,” explains Rosbash. That observation suggests that circadian regulators control the production of genes at two levels: both when genes are transcribed into RNA, and also when that RNA is processed and translated into proteins. His [findings were published](#) January 22, 2013, in the Proceedings of the National Academy of Sciences.

Rosbash has also focused his attention on the dramatic changes that happen every day in the approximately 150 circadian neurons in the fruit fly brain. These neurons are the equivalent of the cells in an area of the mammalian brain called the suprachiasmatic nucleus (SCN), which is responsible for receiving light signals from the eyes and then adjusting the timing of the circadian clock in the rest of the body. When the 20,000 or so neurons in the SCN are obliterated, the clock goes awry. The fly brain, Rosbash says, is a simple model for the more complicated mammalian SCN.

“During the day, the branched ends of certain fly circadian neurons spread out,” he notes. “And then at nighttime, these ends all come together, curling back in. The neuron may go from touching 20 other neurons to just a few, then back to 20.”

[In a paper](#) published in *Neuron* in July 2013, Rosbash reported that the gene *Mef2* links neuron morphology to the circadian clock. Proteins similar to CLOCK and BMAL1, he found, bind to *Mef2* regulatory signals, which—when turned on—reshape brain cells. Rosbash doesn’t yet understand the implications of these daily neuron changes, but he suspects that they’re one more way the internal clock controls physiology. “The clock governs so many processes,” he says. Understanding those processes could lead to the development of ways to entrain the circadian clock for optimal health.

Dusk

The streets of Philadelphia have stilled after a whirl of rush-hour commuters. Street lights flicker on, casting a warm glow on the pavement. Inside Amita Sehgal's lab at the University of Pennsylvania, graduate students hunch silently over their computers as janitors sweep up and down nearby hallways. Inside the lab's cages of *Drosophila*, things are quieting, too—flies have ceased flying and instead pose like statues, unmoving.

Like humans, fruit flies sleep in response to the daily rhythms of their bodies, making them a valuable tool for studying how circadian rhythms control sleep.

"Sleep isn't necessarily the number one best readout of the circadian rhythm when you compare it to molecular methods," says Sehgal, an HHMI investigator. "But it's by far the most obvious and the easiest to observe."

Almost a century ago, scientists discovered that sleep—in humans—syncs with the circadian rhythm. By the 1960s, experiments had shown that even in a constant light or dark environment, people will follow close-to-usual sleep and wake cycles.

To understand the sleep-circadian link, Sehgal is working her way through *Drosophila* chromosomes, one by one, searching for sleep-related genes. In 2008, she [identified the sleepless gene](#) on chromosome 2. More recently, her lab examined chromosome 3, introducing random mutations into genes and then observing which of them changed flies' sleep patterns. [This turned up a gene](#)—which Sehgal dubbed redeye—related to how much, and when, flies sleep.

Sehgal's team has observed that sleepless and redeye mutations do more than change the sleep patterns of flies; they impact their long-term health. Sleepless mutants live half as long as normal flies, and the gene mutation dials up the activity of stem cells in the testes of flies.

Sorting out how sleepless and redeye are linked to the circadian rhythm, however, is tough. When and how much an organism sleeps is affected not only by time of day, but also by how long it's been since its last slumber. The new redeye gene, Sehgal says, may relate to the second influence: the longer a fly has been awake, the higher the levels of redeye. The redeye levels continue to vary over the course of the day, even when fly clock genes are mutated, suggesting that redeye is not controlled entirely by the circadian rhythm.

"Sleep is subject to all sorts of influences," Sehgal says. "What we really want to know is where all these influences converge." The answer may help scientists treat sleep disorders and jetlag, or make shift work easier.



Amita Sehgal is trying to sort out how circadian rhythms affect sleep. Photograph by Kevin Wolf.

Midnight

At the medical intensive care unit at Yale-New Haven Hospital, it's hard to tell when it's the middle of the night. The fluorescent lights still burn brightly, pagers beep, and patients are shaken awake hourly. Inside each person's body, cells struggle to stay on schedule. The metabolic processes that normally wane at night are sputtering, confused by the lights and the noise.

HHMI Investigator Erol Fikrig is all too familiar with this 24-hour chaos—he's an infectious disease clinician at Yale School of Medicine. He's also a researcher who's recently discovered that daily circadian rhythms play a key role in how the human body fights infections.

Fikrig's research group, which studies mosquito- and tick-borne diseases like Lyme disease, dengue fever, and West Nile virus, began exploring whether the timing of insect bites affected how well people fight off disease. "People kept anecdotally telling us, 'I always get bitten by mosquitos at night,' or saying, 'I only get bites right around dusk,'" says Fikrig. "We wondered whether the human immune response is most vulnerable at a certain point in the day."

His lab group measured levels of immune molecules in the bloodstream of mice at different times. One—a receptor called TLR9 that recognizes invading pathogens—fluctuated over 24 hours. During the rodents' sleeping hours, levels of the TLR9 gene plummeted; during their active hours, levels rose again, according to [the group's 2012 report](#) in the journal *Immunity*.



To see whether the changing levels of TLR9 had consequences, the scientists gave mice infections at different time points. The survival rates of the mice, they found, were linked to TLR9 levels at the time of infection. In a subsequent experiment, Fikrig's group found that a vaccine was most effective if given to the mice when TLR9 levels were highest.

"When do you get your flu vaccine? Whenever the clinic is available or you have time," says Fikrig. "None of us put any thought into what time of day we get the vaccine, but maybe we should."

Why would levels of an immune molecule rise and fall throughout the day? It may be taxing to keep the immune system on high alert all the time, Fikrig speculates, and organisms are less susceptible to many pathogens while they're sleeping.

"If you think about people who lived twenty thousand years ago, they were out in their environment all day. At night, when they were resting in their shelter, their susceptibility to

Erol Fikrig wants to understand how immune defenses change from day to night. Photograph by Robert A. Lisak/PR Newswire, © HHMI.

diseases changed,” he says. They were less likely to get a scrape or cut, but more likely to be bitten by a night-loving bug, so the immune system had different challenges to face.

Today, those daily cycles of pathogen risks may be dampened—at least in developed countries—but the circadian cycle of the immune system remains. Fikrig wants to know how to take advantage of this knowledge to help patients.

“Does this make certain patient populations more susceptible to infections at certain times in the day? If so, what can we do about that?” Fikrig asks. “And what happens when a person’s circadian rhythm is off, like when she is jetlagged or in the intensive care unit with the lights on all night?” He’s launched studies to explore those questions.

Dawn

In the moments before the sun’s first rays peek above the horizon, plants already anticipate the light. Sunflowers tilt expectantly east and bean vines unfurl their leaves. Time-lapse movies of plant growth reveal some of the most visually enticing examples of the circadian rhythm. But—as in animals—many of the influences of the internal clock in plants are invisible to the eye because they happen inside cells.

In 2011, HHMI Investigator Xinnian Dong, at Duke University, was studying how *Arabidopsis*—a small, flowering plant common in labs—fights off downy mildew, a fungus-like microbe. She and her colleagues screened thousands of *Arabidopsis* genes and identified 22 gene mutations that weakened the plants’ defenses. Fourteen of the genes, they discovered, had a DNA-binding site that allowed control by circadian proteins.

“We started sampling the expression of these genes every few hours,” Dong says, “and were incredibly surprised to find out that these defense genes have a daily rhythm.” The fungus-fighting genes, she found, were turned on each evening. This pattern of gene expression suggests that the resulting proteins likely accumulate throughout the night.

In retrospect, this makes sense, Dong says. Fungi tend to form spores at night, when it’s cool and damp. In the morning, as moisture on a plant evaporates, the spores dry and are disseminated into a plant’s cells. By rallying the maximum number of defense proteins in the morning hours, plants might effectively fight off downy mildew and related pathogens. Indeed, when Dong’s team exposed *Arabidopsis* to mildew at dusk, the plants developed a more severe infection; the researchers [reported their results](#) in *Nature* in 2011.

Since then, Dong has shifted the focus of her lab to study how plant defenses are linked to daily rhythms. The group has discovered that it’s a two-way street: the circadian clock controls the fluctuating levels of defensive proteins, but an infection with a pathogen can also affect the clock. In fact, they’ve learned that a plant’s circadian control genes begin fluctuating much more strongly when a plant is fighting an infection.

“The clock runs at the same speed, but the changes in gene expression throughout the day are even more extreme,” Dong says. She thinks that this helps the plant maintain its daily rhythms even while excess energy is being used to rid the plant of an infection. “You have this reinforcement of the clock to make sure it ends up back on track,” she says.

As in mammals and flies, sorting out which daily changes in a plant are truly circadian can be tricky. Many plant behaviors—growth speed and direction, for example—are mediated by light, but aren’t truly circadian.

“Ninety percent of the genes in a plant are expressed only at certain times of day,” says Joanne Chory, an HHMI investigator at the Salk Institute for Biological Studies. “But many of those patterns go away if the plant is in constant light or darkness.” That means that the genes are being controlled by the presence or absence of light, not by an internal clock. So outward appearances of the plant behavior can be deceiving.

“Since so many biological processes are regulated by the circadian clock, the big challenge for the future is to study the dynamic interplay among these processes,” says Dong.

Synchrony

Human, mouse, fly, and plant circadian clocks are full of intricacies that make them seem more complex with every discovery. But even in the most basic of circadian clocks, questions still remain.

Since the mid-2000s, HHMI Investigator Erin O’Shea’s lab group at Harvard has focused on studying the circadian clock of cyanobacteria, a type of aquatic bacteria that obtain their energy through photosynthesis. Consisting of just three proteins, the clock, as in plants, lets the organisms anticipate daylight to ramp up their metabolic processes. However, O’Shea found that, rather than being regulated entirely by gene expression, the cyanobacteria clock was controlled over time by the addition and removal of phosphates to or from the three involved proteins.

“The biggest surprise with this clock was how long it can continue keeping time even in constant light,” says O’Shea, who is also HHMI’s vice president and chief scientific officer.

A group of cyanobacteria, without communication between the cells, can remain in synchrony—with each other and the time of day—for weeks. And the simple clocks have a lot in common with more complex clocks: a pulse of darkness, for example, can reset the clock—a phenomenon that also occurs in mammalian cells.

In 2013, O’Shea [published a paper in *Science*](#) that added complexities—and questions—to the cyanobacteria clock. She found that, although the three-part protein clock can maintain a 24-hour cycle in the absence of certain gene fluctuations, circadian regulation of gene expression of clock proteins is required to maintain synchrony.

“There are still a lot of huge, unanswered questions, not just about this clock but about all circadian clocks,” O’Shea says. The answers to those questions, she says, will most likely be found in studies focused on the basic molecular underpinnings that keep the circadian clock ticking in organisms from cyanobacteria to people.

© 2014 Howard Hughes Medical Institute