For the past 25 years, a pair of Dartmouth researchers has been trying to figure out how a fungus tells time. Along the way, Jay Dunlap, Ph.D., and Jennifer Loros, Ph.D., have helped to explain one of biology’s fundamental mysteries—how organisms, including humans, have adapted to the 24-hour cycle that we call a day.

During the time it takes for the Earth to rotate on its axis once, the average person experiences a host of physiological changes. Your body temperature rises from a low of about 97 degrees to a high of about 99 degrees, then falls again. Your short-term memory peaks just after you wake up, while your athletic performance improves in the late afternoon. And after midday, as drowsy workers head home for a siesta, your body temperature falls from a mid-afternoon high of about 99 degrees, then rises again, to fuel growth. The fungus spends most of the day spreading down the length of the tube, a few millimeters per hour. But around midnight, it starts to lay down cells that can produce spores. Over the next several hours, as Neurospora continues to grow horizontally, these cells will simultaneously grow vertically, producing orange spores that rise above the mass of the fungus on the bottom of the race tube. By mid-morning, the spores have matured and are ready to be picked up by a breeze. At this point, Neurospora stops producing spores, growing only horizontally until the clock again strikes midnight.

Dunlap now expounds enthusiastically about this question, but circadian biology was not a field that he originally intended to enter. As an undergraduate, he majored in oceanography. He then enrolled in graduate school at Harvard, planning to get a degree in biochemistry and apply it to marine life. Early in his study of biochemistry, he came across tiny creatures called dinoflagellates. Some species of these single-celled organisms can produce flashes of light—something like seafaring fireflies. Their bioluminescence is the result of a reaction between a number of proteins, and these proteins are present in much wider contexts. And after midday, as drowsy workers head home for a siesta, your body temperature falls from a mid-afternoon high of about 99 degrees, then rises again. This pair of Dartmouth researchers has become increasingly wise to the ways of the circadian clocks that control the daily routines of virtually every organism on Earth—from human beings to the simple fungus that they study.
The DMS researchers concluded that circadian clocks work as a negative feedback loop. An example often given of a negative feedback loop is a household thermostat. Too much heat causes the furnace to turn off, which allows the temperature to drop, which makes the furnace turn back on—thus stabilizing the temperature. Some scientists still weren’t convinced of the merits of circadian biology and didn’t think studying a fungus could lead to a better understanding of circadian clocks in humans. There was another factor in the decision, as well. “It was the only job I got,” Dunlap says. “That made the choice easy.”

For a time, Loros remained in California to finish her dissertation. But that fall, she married Jerry Dunlap, Ph.D., soon joined the lab. “When I came to Jay’s lab, I wasn’t necessarily thinking that I would be working with postdoc Benjamin Aronson, Ph.D., in Neurospora. I had earlier identified a Neurospora gene called frq (frequency). Feldman had outlined three bold lines of research. He wanted to know how circadian clocks work at the molecular level. How are the components of Neurospora’s behavioral clock tied together, white collar-1 and white collar-2 form a transcription factor for frq. As a result of this work, Neurospora’s circadian clock is now known to be regulated by a negative feedback loop. An example often given of a negative feedback loop is the regulation of house- hold thermostats. If the temperature gets too high, the thermostat turns it off; this reduces the temperature, which makes the furnace turn back on to produce more heat, and so on. The expression of frq expression is central to the proper functioning of the clock.”

Based on their findings in Neurospora, and the work of other researchers in fruit flies, Dunlap and Loros inferred that the circadian clock works as a negative feedback loop. An example often given of a negative feedback loop is the regulation of household thermostats. If the temperature gets too high, the thermostat turns it off; this reduces the temperature, which makes the furnace turn back on to produce more heat, and so on. The expression of frq expression is central to the proper functioning of the clock.”

Working together, Dunlap and McClung succeeded in cloning the frq gene, opening up new ways of investigating the effect of the gene in the circadian clock. The breakthrough turned them a spot in the prestigious journal Nature. “It was tremendously exciting,” McClung says.

“Our goal was to understand the molecular basis of circadian rhythms; the leaves of this plant, Cassie corymbosa, are open during the day (left) but curl up at night. Over the past 15 years, Dunlap and Loros have continued to add more pieces to this puzzle, resulting in a detailed understanding of Neurospora’s circadian clock. Along with frq, two other cellular components form the heart of the negative feedback loop—the proteins white collar-1 and white collar-2 (named for the effect that mutations in these genes have on Neurospora’s appearance). Together, white collar-1 and white collar-2 form a transcription factor for frq, meaning that when they bind to that gene, they turn it on, leading to the production of frq RNA.”

In the hours after midnight, this transcription factor gets to work, prompting the expression of frq. By mid-morning, there’s an abundance of frq RNA available in Neurospora cells. Eventually, the frq RNA is translated into protein. These proteins provide negative feedback by blocking the transcription factor from binding to the frq gene. As a result of this obstruction, the production of frq RNA slows down in the late morn- ing and early afternoon. The frq RNA that has already been produced continues to be translated, which means that protein levels reach their peak in the mid-afternoon or early evening—hours after

Worshipping a postdoc, Benjamin Aronson, Ph.D., Dunlap and Loros discovered that the expression of frq turned up at some points during the day and down at others, following a regular 24-hour cycle. Simplified, there are two basic steps in gene expression: transcription, in which DNA is transcribed into RNA, and translation, in which RNA is translated into proteins. The researchers found that frequency pro- teins limit the production of frq RNA—in other words, the translation part of the process feeds back to regulate transcription. When frq expression did not fluctuate—for example, when Neurospora was manipulated so that frq RNA levels remained constant—the fungus no longer followed the 24-hour cycle of horizontal growth and spore production. Instead, it produced spores constantly. Clearly the cycling of frq expression was central to the proper functioning of the clock.”

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Dunlap wondered how these organisms could possibly know to expend energy to generate light only at night. No one had explained this phenomenon. It was clear that this was a problem no one had any clue as to how it worked,” Dunlap says. “As far as I could tell, they weren’t even pursuing it in the ways in which you’d have to pursue it in order to get an answer. So I saw it as a great problem.”

Dunlap decided to take on circadian rhythms, but in Neurospora, an organism that is widely used for genetic studies. He went on to a postdoctoral position in the lab of Jerry Feldman, Ph.D., at the University of California at Santa Cruz. Feldman had earlier identified a Neurospora gene called frq (frequency). Feldman found that strains of Neurospora with a mutated form of frq display abnormal circadian rhythms, indicating that the gene must play some role in the organism’s clock. As a postdoc, Dunlap encountered new tools made available by the revolution taking place in molecular biology—tools that made it easier to dissect the inner workings of cells. It was also while he was a postdoc that he met Loros. Jennifer Loros was a graduate student at UC- Santa Cruz, and, like Dunlap, she started her scien- tific career with a very different focus from circadi- an rhythms. “I’m a gardener,” she says. “That’s ac- tually how I got into science.” She’d planned to study hormones in plants but realized “I can’t stand a wall of leaves with my first Ph.D. project and end- up changing laboratories to work in circadian rhythms. And I’ve been doing it ever since.”

Early in 1984, Dunlap took a position as an as- sistant professor at Dartmouth Medical School. “They were looking for someone who was doing something different,” he recalls. At the time, circadian rhythms certainly fit that description; in fact, some scientists still weren’t convinced of the merits of circadian biology and didn’t think studying a fungus could lead to a better understanding of circadian clocks in humans. There was another factor in the decision, as well. “It was the only job I got,” Dunlap says. “That made the choice easy.”

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In 1994, Dunlap and Loros published their results in another top journal, Science, noting similarities between their findings and the circadian clocks of fruit flies. Studying a fungus was by then looking like a very promising way to find out exactly how circadian clocks work in more complex organisms.
Say a Neurospora cell catches a morning flight from Paris to New York. When it arrives in mid-morning, the cell feels as if it’s mid-afternoon; if it had stayed in Paris, its frequency proteins would be starting to decline.

But the extra hours of daylight in New York adjust its circadian clock.

If a cell catches a morning flight from Paris to New York, it feels as if it’s mid-afternoon in Paris. The cell’s circadian clock is adjusting to the extra hours of daylight.

The amount of frq RNA goes into decline. Over time, frequency proteins are broken down by enzymes, diminishing their numbers. And with no remaining reservoir of frq RNA waiting to be translated, protein levels continue to decline.

The graph below shows fluctuations in Neurospora's relative levels of frq RNA, frequency proteins, and white collar-1 proteins over the course of a little more than one circadian cycle; day and night are indicated by the white and black portions of the bottom bar.

This set—if it consistently followed a 22-hour growth cycle! At first, it might not make much difference. But within about a week, the fungus would produce spores at noon, when the sun is most intense. The spores are vulnerable to ultraviolet rays and so less likely to survive if left to bake in the sun, which would reduce Neurospora’s ability to spread.

Producing spores costs Neurospora in energy expenditure, but the spores are an essential part of its life cycle. So in every sense that, over evolutionary time, Neurospora has developed a rhythm that enhances the odds that spores survive. Dunlap cautions that this explanation for Neurospora’s development pattern hasn’t been proven experimentally. But he notes that studies in other organisms, including plants and cyanobacteria, have shown that adhering to a cycle in tune with the Earth’s rotation does provide fitness advantages.

Dunlap, at left, is a member of the National Academy of Sciences, the premier U.S. scientific body. He and Loren—who is pictured above with their dog, Fionn—collaborate 24/7. They were married in ’84, she became a postdoc in his lab in ’85, and they’ve shared lab space ever since, though they’re both now independently funded investigators.

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Circle-wise

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age occurs, resetting the clock might keep a cell from dividing at a time when a mutation is likely to result; thereby preventing mutations that could lead to tumor growth. Earlier this year, postdoc Joshua Gamsby, Ph.D., reported similar results in mouse cells, proving that in mice, as in Neurospora, DNA damage can reset the clock.

These findings add to the growing recognition of the relationship between the circadian clock and cancer. “We are, as a field, now understanding that the clock is very important in a number of different cell-cycle events,” says Loros, “and that misregulation in clocks often results in cancer.”

In one study, mice lacking the gene Per2, a key clock component in mammals, were more likely to develop tumors. Another study, done in Denmark, found that women who work at night had a greater risk of breast cancer than women who work day shifts. And a number of studies have shown that timing chemotherapy to take advantage of circadian rhythms might improve outcomes.

“Many people don’t want to leave, because it’s such a nice environment!”

Providing the researchers in their lab with the freedom to develop their own ideas has been a conscious decision for Dunlap and Loros. “We give people an enormous amount of independence,” Loros says. “We rarely tell people what to do,” Dunlap agrees. “We’ll suggest projects when they get here if they don’t come in with something they want to do, but almost always within some months they have either developed entirely their own ideas or developed additional ideas that are their own.”

Loros points out that much of their own success is due to the hard work of these up-and-coming researchers. “It’s been very gratifying for all the people I’ve worked with over time,” she says. “Good science is very often collaborative, so we’ve been lucky.”

Of course, each one’s most important collaborator has always been the other. “In science, people constantly talk about how it’s important to have a built-in colleague,” she says. “I feel very grateful for all the people I’ve worked with over the years.”

Today the study of circadian rhythms no longer qualifies as “something different,” in part due to all the researchers who have passed through the Dunlap-Loros lab. Bob Froehlich, a senior scientist at a biotech company, also credits Dunlap and Loros for helping develop new generations of scientists. “The training in that lab set me up well for what I’m doing now,” he says.

Giles Duffield, Ph.D., spent time in their lab as a postdoc before leaving to join the biology faculty at Notre Dame. “When people leave the lab, they know how to do good science,” he says. “Of course, he adds, many people don’t want to leave, because it’s such a nice environment!”

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