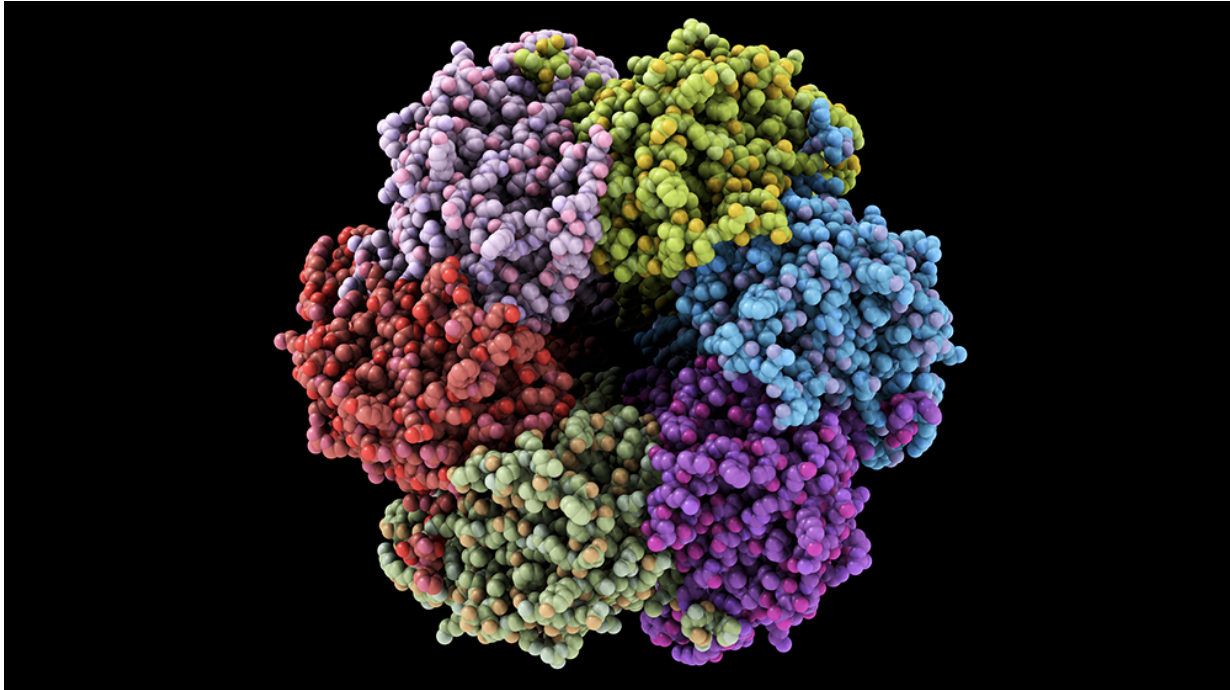


# How the Body's Trillions of Clocks Keep Time

Cellular clocks are almost everywhere. Clues to how they work are coming from the places that they're not.



*Laguna Design/Science Source*

Individual proteins, like this protein found in blue-green algae, help regulate circadian rhythms.

*By Veronique Greenwood*

[Carrie Partch](#) was at the tail end of her postdoc when she made the first discovery. The structural biologist was looking at a database of human proteins, noting those that shared a piece with the ones she'd been studying. "I was just sort of flipping through it thinking, 'I should know all of these,'" she recalls. "Then this one came up, and it had a different domain architecture than I'd ever seen." She looked further into the protein, called PASD1, whose function was unknown. She found that among the few proteins it resembled was one called CLOCK. And that made her sit up straighter — because CLOCK is at the heart of a very large, mysterious process.

Not that long ago, as Partch knew, it had become clear that nearly every cell in nearly every tissue in the body keeps time. Every 24 hours, responding to a biochemical bugle call, a handful of proteins assembles in the cell's nucleus. When they bind to each other on the genome, they become a team of unrivaled impact: Under their influence, thousands of genes are transcribed into proteins. The gears of the cell jolt into motion, the tissue comes alive, and on the level of the organism, you open your eyes and feel a little hungry for breakfast.

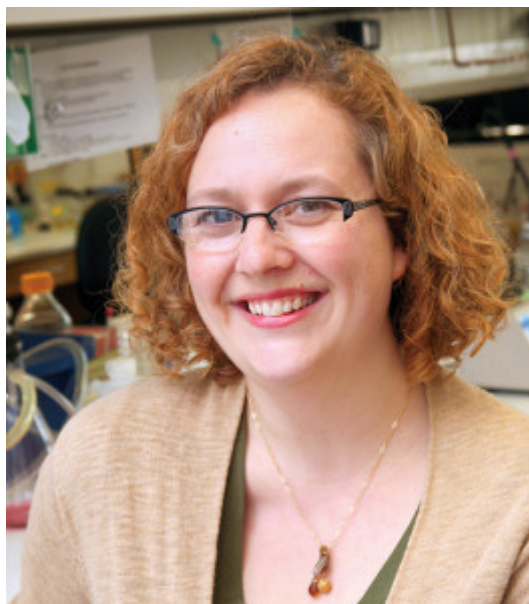
These timekeeping protein complexes, which take some of their cues from a part of the brain that responds to light and darkness, are known as circadian clocks. By some estimates, they regulate the expression of 40 percent of the genes in the body. Researchers are accumulating evidence that circadian clocks have deep effects on everything from fetal development to disease. Circadian clocks are so ubiquitous, and so important to the function of individual cells, that biologists whose research doesn't overtly connect to a clock are becoming aware of how it might impact their work. "More and

more they are stumbling into clock components,” said [Charles Weitz](#), a molecular biologist at Harvard Medical School. “It doesn’t surprise me.”

Very few cells lack a clock, but they include biologically compelling examples like embryonic stem cells and cancer. In an effort to discern how the molecular clock works — and why, sometimes, it appears to stop — Partch decided to look closer at PASD1. As she and her colleagues [recently revealed in a paper in \*Molecular Cell\*](#), PASD1 may be a switch that explains how cells as different from each other as cancers and sperm precursors escape the daily rhythms that govern the trillions of other cells in the body. It gives researchers a deep look at the secrets of how the cell ticks.

### The Clock the Cow Found

The daily cycling of plants and animals has been a source of fascination for millennia, but it wasn’t until about 50 years ago that research into the underlying biochemistry began to take off. Many people trace the field’s founding to [a meeting at Cold Spring Harbor](#) in the summer of 1960, where researchers brainstormed ideas about what might cause circadian rhythms and devised experiments to test their theories. Over the ensuing three decades, researchers identified mutant creatures with abnormal daily cycles — fruit flies, hamsters, yeasts and others — and began to uncover the genes required for a normal rhythm. Studying flies whose natural cycle was 19 or 28 hours, or who had no discernible rhythm, led clock pioneers Ronald Konopka and Seymour Benzer to [discover the first family of key clock genes](#), which they named *per*, in 1971, and whose levels we now know to rise and fall through the day. Just a year later, researchers reported that a tiny patch of cells in the brain called the suprachiasmatic nucleus was [necessary for a 24-hour circadian rhythm](#) in mammals.



*Courtesy of Carrie Partch*

Carrie Partch has discovered a protein that interferes with circadian rhythms.

Yet for many years it was not clear how pervasive the rhythm’s effects were — how deeply it affects everything in the body. In 1988, [Ueli Schibler](#), now a professor of molecular biology at the University of Geneva, was studying transcription factors, cellular actors that control the transcription of genes into proteins. One factor in particular, isolated from rats by a Canadian postdoc, seemed to be quite

powerful. Together they published their discovery in the journal *Cell*. Three months later, however, a student named Jérôme Wuarin took over the project. He soon approached Schibler with some disturbing news.

“You’ve got to retract this paper,” Schibler recalls Wuarin saying. “It’s all fake. It doesn’t exist.” When Wuarin performed the isolation, the transcription factor had failed to appear. Schibler, taking his concerns seriously, tried the procedure himself. He found the transcription factor easily.

After a number of weeks, Wuarin realized why he couldn’t find it himself: He and the postdoc had been performing the isolation at different times of day. The postdoc, a late riser, usually arrived around 11 a.m., killed the rats, and had the transcription factor in hand by midafternoon. “But [Wuarin] was a farmer’s son,” Schibler explains. “He got up at 5, milked the cows, then came to lab and killed the rats at 7. And at that time, this protein’s just not there.”

It’s now known that every day, this transcription factor’s levels start at almost nothing, making it impossible to detect in the morning, and then rise 300-fold, making it easy for the postdoc to find in the middle of the day. Schibler notes wryly, as an aside, that in all the years since, no one has ever found a protein that oscillates more wildly. It was just their luck.

After this discovery — that the circadian rhythms of the researchers and the circadian rhythms of the rats interacted to make the protein appear to disappear — Schibler turned to studying the daily rhythm and its control of transcription more closely. In 1998, he and colleagues [found something unexpected](#). For years, the cells of the suprachiasmatic nucleus were thought to be alone in having their own clock, controlling all the rhythms in the rest of the body remotely. But Schibler and colleagues found that the brain wasn’t required for a rhythm; nor really was a body. Two kinds of rat cells, grown in dishes for generations, would rhythmically express genes all on their own. The team’s work joined a handful of [other studies](#) suggesting that body clocks were more widely distributed than people had thought.

Since then, [liver cells](#), [heart cells](#), [lung cells](#) — in the words of Charles Weitz, “just about every tissue we’ve looked at” — have turned out to beat their own time, in addition to taking cues from the suprachiasmatic nucleus. “Almost every cell in our body has a circadian clock,” said [Satchin Panda](#), a clock researcher at the Salk Institute. “It helps every cell figure out when to use energy, when to rest, when to repair DNA, or to replicate DNA.” Even hair cells, for instance, divide at a particular time each evening, Panda has found. Give cancer patients radiation therapy in the evening rather than in the morning and they might lose less hair.

Researchers have spent the past 15 years untangling the molecular components of these peripheral clocks, as they’re known. A big step forward came in 2004, when a team led by [Joseph Takahashi](#), now a professor at the University of Texas Southwestern, developed mice with a glowing PER protein. When PER is expressed, cells from these mice are bright; when it’s not, they’re dark. This advance has enabled [studies](#) that track the clock’s cycling in myriad different tissues and circumstances.

Researchers have found that peripheral clocks are based on CLOCK and a protein called BMAL1, as is the clock in the suprachiasmatic nucleus. Clasping each other tightly, this pair attaches to the genome and recruits other proteins to start the transcription of nearby genes, including *per*. Many of these genes are behind certain physiological rhythms — the production of liver enzymes around mealtime, for instance, and the daily rise and fall of blood pressure.

But some proteins, including PER, serve as counterbalances. As PER and its partners gradually build up in the cell over a period of 12 hours, they inhibit the activity of CLOCK and BMAL1. Over the next

12 hours, the counterbalances are slowly degraded, and CLOCK and BMAL1 surge back. Just before dawn and just before dusk, [John Hogenesch](#), a chronobiologist at University of Pennsylvania, has found, there are “rush hours” of gene expression, perhaps the body preparing for the different demands of surviving in the light and in the darkness.

It’s a tidy, self-governing system, and it’s tempting to call it ubiquitous. But these studies have revealed too that not everything has a clock. Embryonic stem cells, which can develop into almost any cell type, don’t keep time. The testes, almost alone among the organs that have been tested, don’t seem to have a clock either. And many cancer cells do not keep a regular rhythm. What could these things have in common? This is where Partch’s discovery comes in.

## How the Clock Stops

One of the first things Partch learned about PASD1 was that it appears in very few tissues. But the ones where it does are intriguing: the testes and cancers. When Partch became a professor at the University of California, Santa Cruz, she and her students began adding PASD1 to cells equipped with glowing PER. They found the cells’ usual light was damped down to a faint glimmer, indicating that PASD1 was interfering with the normal operation of the clock. And the more PASD1 they added, the dimmer the cells were.

Next, Partch and her students grew cells with glowing PER and got all the cellular clocks synchronized. The glow would get brighter and dimmer like a sine wave with a 24-hour period, with defined peaks and troughs for as long as the cells stayed synchronized. Partch then caused some of those cells to produce PASD1. In these cells, the glow became more of a wobble than a wave — the peaks low and the troughs shallow — and very soon it faded away. The cells could not maintain their rhythm.

The team is still working to pin down exactly how PASD1 calls a halt to the cells’ cycling. But one specific part of the protein gives them a hint. This section of PASD1 looks like a part of CLOCK that is absolutely essential for circadian rhythms. “But no one still to this day knows quite exactly what it does,” Partch said. She hopes that by understanding how the key piece of PASD1 works — perhaps, for instance, it binds to BMAL1 itself and keeps CLOCK from doing so — they can learn the role of this key piece of CLOCK.

So far, the work has confirmed Partch’s initial hunch that PASD1 would stop the clock. And it suggests that in the tissues where PASD1 is present, it is part of the reason why the cells don’t oscillate. That finding opens the door to deeper questions: With the clock directing so many aspects of cellular behavior, and with mutations in clock genes leading to illness — they’ve been fingered in cancers and metabolic disorders — why would some types of cells lack a clock or have a weakened one?

“It seems like there’s some really interesting and still unexplored connection between some perfect pluripotency,” meaning the cellular ability to develop into any cell type, “and running a clock,” Partch said. She recounts experiments from Kazuhiro Yagita’s lab, in which embryonic mouse stem cells are spurred into development. “At first, it’s like come on, come on, no ticking, no ticking... and then, at some point in the differentiation of these cells, the clock comes on.” When the process is reversed, the clock turns off.

The lack of a clock in stem cells may be because the precise genes controlled by the clock vary so much from tissue to tissue, Partch speculates. Work from Charles Weitz’s lab has shown that liver and heart tissues share only 8 to 10 percent of genes that oscillate daily, for instance. “[Stem cells] have to be everything and nothing at once,” Partch said. “Maybe in a cell that doesn’t know what it

is yet, it's not ideal" to have a clock. It's a notion that could encompass the testes, where mature sperm are far outnumbered by stem cell precursors, and where PASD1 has been spotted. She has yet to look for PASD1 in other stem cells.

In cancers, the protein's other known hangout, the reasons for its presence are likely to be different. "It may be the reason why the clock is not operational in most solid tumors," said Hogenesch, who was not involved in the work. "If you're a tumor, and you want to keep dividing and dividing and dividing, maybe you don't want to be confined to dividing at one time of the day. Maybe there's an evolutionary advantage — to tumors at least — to disrupt the clock so they can divide whenever they have sufficient resources rather than being nudged and nuanced to divide at a particular time of day." Partch's group found that interfering with PASD1's production in two cancer cell lines made their oscillations stronger and more regular. That suggests that future work should look to see whether knocking PASD1 down might also rein in the cancer cells' out-of-control reproduction.

Ultimately, the research should illuminate something more fundamental. "Understanding how PASD1 interferes with clock function lets us know how the clock is working," Partch said. She and her team are also realizing that just as the clock affects far more processes than were at first evident, PASD1 may be doing more than just interfering with CLOCK and BMAL1. But that work will come, with time.

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