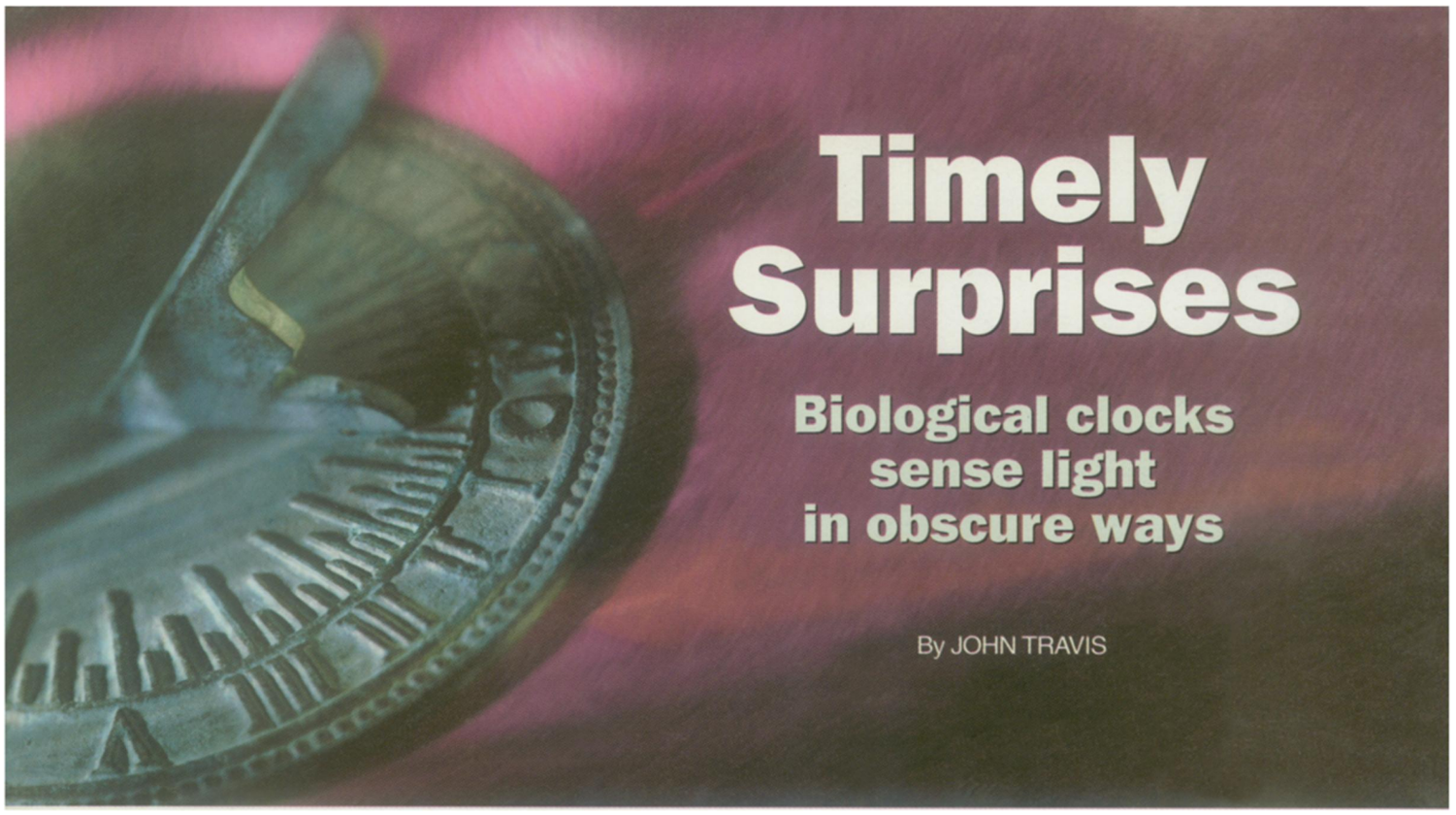


Timely Surprises

Biological clocks sense light in obscure ways

By JOHN TRAVIS

When a New Yorker jets off to Los Angeles or Tokyo, it takes just a few seconds to adjust a wristwatch to local time. But as sufferers of jet lag know all too well, the body's internal clock isn't nearly so quick to adapt. It usually takes a few days for a person's biological clock to synchronize with the dusk and dawn of a new time zone.

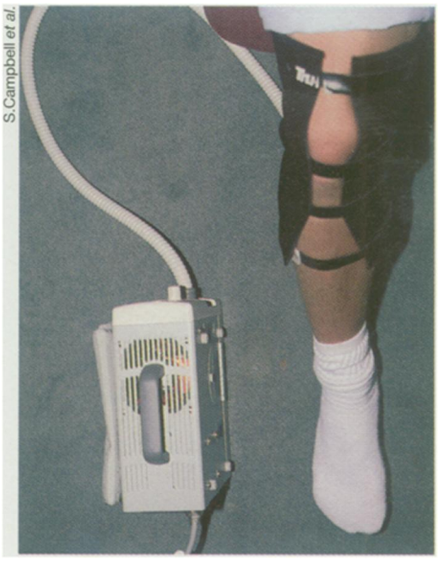
The lethargy of jet lag sets in during this adjustment period because this internal clock has a profound influence on the human body. It governs when we feel we should sleep or be active. It establishes daily, or circadian, rhythms in characteristics as diverse as hormonal concentrations and body temperature.

Fortunately for travelers, the human biological clock uses environmental cues, primarily light, to continually match its time to that of the outside world. Yet mysteries remain about this entrainment, and two new studies have added to them.

In one report, two researchers counter conventional wisdom with evidence that light shone on skin, rather than the eyes, can reset the biological clock. In the second, scientists suggest that the clock's response to light depends not upon the well-known light-sensing molecules of the eyes but rather on proteins related to plant photoreceptors.

Neither study is definitive, and skepticism remains about both conclusions. Still, scientists who study biological clocks and the circadian rhythms they generate have of late encountered a fair share of surprises. Last year, for example, studies suggested that fruit flies may have independent biological clocks throughout their tissues rather than a single clock in their brains (SN: 12/06/97, p. 365).

"We keep finding, almost every week, that there are remarkable things we don't know about our circadian system. The lesson is we should keep an open mind," says Thomas Wehr of the National Institute of Mental Health in Bethesda, Md.



S. Campbell *et al.*

This device sends light through fiber optic cables to a blanket wrapped around a person's knees.

Wehr's open mind has been tested of late by what may be the most bizarre result ever described in biological clock research. In the Jan. 16 SCIENCE, Scott S. Campbell and Patricia J. Murphy of Cornell University Medical College in White Plains, N.Y., report that light focused on the backs of people's knees reset their biological clock as readily as light shone on the eyes.

If true, this finding might make light therapy, now used by shift workers and people with jet lag, insomnia, or other sleep disorders, much more practical. "You may be able to do it while you're asleep," says Campbell. "Compliance is a major issue [with light therapy]. People don't have time to sit in front of a light box for a couple of hours."

Campbell and Murphy's unusual experiment is in fact the offshoot of a study conducted by Wehr more than a decade ago. Wehr and his colleagues had tested whether light therapy could offer any relief to people with seasonal affective disorder, a depression stemming from the shorter days—and fewer hours of sunlight—of winter.

Exposing their eyes to intense light proved helpful to most of the subjects, but even when their eyes were shielded and the light shone just on the skin, 2 of the 10 people still reported feeling better. Campbell recalls that when Wehr discussed the experiment at meetings, he frequently suggested that someone should repeat the study. "We finally got around to doing it," laughs Campbell.

Instead of relying on a subjective assessment of whether a person feels better, the investigators examined two well-studied physiological measures of the human biological clock—body temperature and saliva concentrations of the hormone melatonin. Both body temperature

Volunteers sit at a table cloaked in black cloth to prevent outside light from reaching their knees.

and melatonin concentrations rise and fall with a circadian pattern, and light provided to a person's eyes can advance or delay those cycles.

To test whether light directed at other body locations can produce similar changes, Campbell and Murphy borrowed a device normally used to treat newborns with jaundice. This device, called the Billiblanket, consists of a halogen lamp that feeds light into 2,400 fiber-optic cables, each one ending inside a woven pad. The fiberoptics separate the light from the heat of the halogen lamp, which itself could influence a person's internal clock. Participants in the study had these pads secured to the back of their knees and received 3-hour light exposures from them. To prevent the light from reaching their eyes, participants sat with their legs below a table draped with an opaque cloth.

In tests of 15 people, Campbell and Murphy found that light on the back of the knees matched the clock-altering ability of light directed into the eyes. The light-delivering pads efficiently advanced or delayed a person's biological clock, as measured by body temperature and melatonin saliva concentrations.

Those unexpected results leave most biological-clock scientists incredulous. "I'm very skeptical," says Michael Menaker of the University of Virginia in Charlottesville. "I'd like to see [the study] repeated a couple of times by other laboratories. It's so out of the ordinary."

How could light hitting the back of knees affect a biological clock? Presumably, says Campbell, the light is falling on some unknown photoreceptor there that is tied into the circadian system. One researcher, Dan A. Oren of Yale University, has even suggested that light penetrates the skin and strikes bloodborne photoreceptors that convey their signals to the brain's biological clock. The molecule hemoglobin might serve that role, he says.

The study by Campbell and Murphy underscores an enduring mystery about the biological clocks of people and other mammals. While scientists have long recognized that light can reset internal timepieces, they're still at a loss to explain how the circadian system perceives the light.

The obvious suspects, light-sensing opsin molecules in the rod and cone cells of eyes, appear innocent. Consider a well-studied mutant strain of mice that suffers retinal degeneration. "These mice lose the layers of the retina that contain rods and cones. After a few months, they're totally blind. But their response to light, as far as the circadian clock goes, is absolutely normal," says Aziz Sancar of the University of North Carolina School of Medicine in Chapel Hill.

Despite the recent back-of-the-knee study, eyes still seem to be the primary route by which the mammalian circadian system senses light. If scientists cut the optic nerve conveying information from the retina to the brain, mice lose their normal circadian rhythms. To resolve this seemingly contradictory evidence, investigators argue that retinas must contain light-sensitive proteins other than the traditional opsins.

"There's something in the retina that's not a rod and not a cone but is a specialized circadian photoreceptor," says Menaker.

Sancar thinks he has found that photoreceptor. He normally studies the DNA-repair systems of microbial cells, particularly light-activated enzymes called photolyases that repair genetic damage caused by ultraviolet radiation. Although people weren't thought to possess such enzymes, Sancar and other scientists recently found two human genes encoding proteins with a strong resemblance to microbial photolyases. These proteins demonstrated no DNA-repair activity, however.

Sancar then learned that other scientists had discovered similar proteins, named cryptochromes, in plants. There, they serve as photoreceptors, governing a plant's circadian response to blue light. "That was an important conceptual clue for us," he says.

Sancar and his colleague Yasuhide Miyamoto have now found that the human cryptochromes, designated CRY1 and CRY2, are made in retinal cells. Moreover, CRY1 is also made in the suprachiasmatic nucleus (SCN), the brain region thought to harbor the master biological clock in mammals. Production of CRY1 in that brain region rises and falls with a daily rhythm. Finally, CRY1 is abundant in skin tissue.

From those pieces of evidence, Sancar

contends that the cryptochromes may explain how eyes—and the skin, if the back-of-the-knee study proves correct—senses light for the mammalian biological clock.

Circadian scientists express both fascination and caution about Sancar's work. "I think what's missing is functional evidence that [CRY1 and CRY2] play a role, but it's very intriguing that they're in the right places. They're certainly a very new and interesting candidate that we hadn't been thinking about," says Joseph S. Takahashi of Northwestern University in Evanston, Ill.

Noting that novel opsins are continually being discovered, scientists warn that they may have simply missed an opsin in the retina. "When you look for opsins and don't find them, what it can mean is that you don't have the right antibodies, you don't have the right techniques, and so on. I would say [the identity of the circadian photoreceptor] is still very much up in the air at the moment," comments Menaker.

Sancar acknowledges that he has not yet proven that the cryptochromes are the circadian photoreceptors, but he suggests that mice genetically engineered to lack the proteins may resolve the issue. Sancar has already made a strain of mice lacking one of the cryptochromes. The circadian behavior of these mice is under study by Takahashi, an investigation that may take several months.

"I think everyone is waiting for us to test the knockout mice and see that they're visually okay but circadian blind," says Sancar. □

Piecing together the clock's workings

Even while they are struggling to explain how biological clocks perceive light, investigators have made dramatic advances in identifying the cogs and gears of these internal timepieces and in understanding how they interact to create a daily cycle. In just the last two months, scientists have described a number of crucial new biological clock components.

"It's a great time for the field. It's really booming," says Michael Rosbash of Brandeis University in Waltham, Mass.

Through studies of fruit flies and mice, scientists had previously identified several key clock components, proteins called PER, TIM, and CLOCK (SN: 5/17/97, p. 300). Several research teams have now independently identified a partner for CLOCK and have suggested how the two proteins join together to regulate the production of PER and TIM. Furthermore, scientists have found that PER and TIM also regulate their own production by interfering with the actions of CLOCK and its partner. While the details may vary from organism to organism, this core feedback loop appears to lie at the heart of the biological clock mechanism in organisms ranging from yeast to humans.

Investigators who discovered CLOCK last year noted two telling features of the protein. It has both a DNA-binding region and a site with which it appears to bind to another protein. Those attributes prompted speculation that CLOCK, when linked to a second protein, turns on the genes that encode PER and TIM.

Five reports, appearing in the May 12 Proceedings of the National Academy of Sciences, the May 29 Cell, and the June 5 Science, provide various pieces of evidence that together confirm the speculation. For example, Rosbash and his colleagues describe in Cell their discovery of the fruit fly version of CLOCK and their subsequent identification of another fly protein, which they name CYCLE, that can bind to CLOCK. They found both proteins by identifying mutant genes that cause fruit flies to lack circadian rhythms. As expected, mutations in the genes for CLOCK or CYCLE eliminated any activity by the genes for PER and TIM.

In other experiments, research groups led by Christopher A. Bradfield of the University of Wisconsin Medical School in Madison, Steve A. Kay of Scripps Research Institute in La Jolla, Calif., and Charles J. Weitz of Harvard Medical School in Boston have established that CLOCK and its partner bind to a specific DNA sequence called a promoter. The resulting activity of this specific promoter increases the activity of the genes for PER and TIM but not of other genes. Closing the loop, Kay and his colleagues also report that PER and TIM, once they enter the nucleus, block CLOCK's ability to activate their genes, perhaps by binding to CLOCK or its partner.

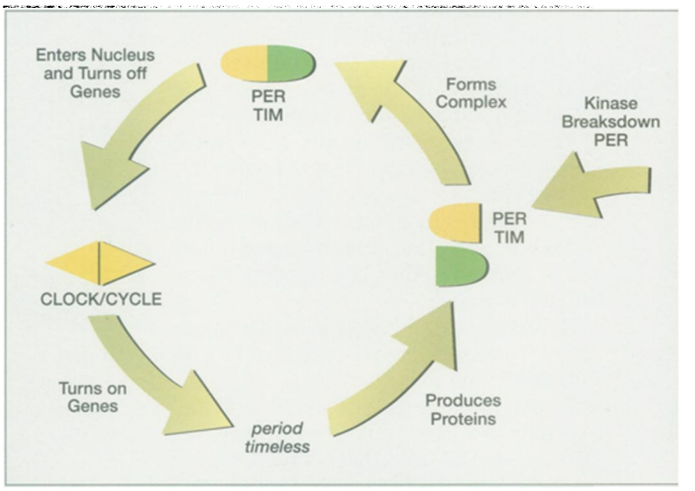
Models based on these four proteins alone seemed unable to generate a clock cycle nearly 24 hours long, researchers agreed. "We now have the component that's missing," contends Michael W. Young of Rockefeller University in New York.

That component is a protein, an enzyme called a kinase, that chemically modifies PER proteins, thereby targeting them for destruction. Young and colleagues, who describe their work in the July 10 Cell, identified the kinase by hunting down the mutant gene in another strain of flies having no circadian rhythms.

While CLOCK and its partner drive the production of TIM and PER in a cell's cytoplasm, the kinase apparently prevents PER from accumulating. After a certain amount of time, however, TIM proteins become abundant enough that they grab PER proteins before the kinase gets to them. The PER-TIM complexes are resistant to the kinase, leaving them free to travel into the nucleus, where they shut down the activity of the two protein's genes. The PER-TIM complexes then somehow disappear, allowing CLOCK and its partner to switch on the genes for PER and TIM again.

The delay introduced by the kinase is crucial, says Young. "In the absence of the kinase, you don't have a clock. In the presence of it, you have very tight control of the production of the negative regulators [PER-TIM complexes]. The kinase provides the lag necessary to make this [loop] oscillate," he explains.

Investigators caution that more components of the biological clock may still be found and that other feedback loops or delays are probably involved in fine-tuning the oscillation driven by CLOCK, PER, TIM, and the other known proteins. "We may now be glimpsing the core of the circadian clock, but we've only begun to scratch at the surrounding loops. For clock watchers, this cannot be considered the beginning of the end, but it might be the end of the beginning," says Jay C. Dunlap of Dartmouth Medical School in Hanover, N.H., in the June 5 Science. —*J. Travis*



In the current model of the fruit fly biological clock, protein complexes formed by CLOCK and CYCLE bind DNA, leading to production of the PER and TIM proteins. A kinase destroys PER, but eventually enough TIM builds up that the two proteins form complexes before PER is destroyed. The complexes inactivate the CLOCK/CYCLE complexes. Over time, the PER/TIM complexes are destroyed, freeing the cycle to start again.