biotechnology because this is the most promising and able to bring back economic support to the whole system," explains Ismael Clark, president of the Cuban Academy of Sciences. Rank-and-file scientists seem to share this outlook. "It's a very strategic area. We don't complain," say University of Havana physicist Ernesto Estévez.

**After the revolution?**

For scientists who believe in Cuba's biotech dream and are determined to remain in the country, the elusive goal is breaking into markets in developed nations. But Cuba faces many obstacles, including the high costs of getting approval to sell products in such countries. Cuba is "weak" in quality-control standards and marketing skills and has only recently begun applying for patents, notes Mikael Jondal of the Karolinska Institute in Sweden, who 2 years ago served on a European fact-finding mission to Cuba. Cuban leaders respond that their labs now adhere to international standards for quality control and clinical trials.

A deal that Cuba inked with a Canadian company in 1994 offers reason for optimism but also shows the challenges the country faces. The firm, York Medical Inc., is acting as a partner to get Cuban products through Canada's regulatory hoops and then license them to drug companies. But of the five products it tapped as most promising, one—streptokinase—has lost major ground to another drug, TPA, says CEO David Allan; and a method for selecting the best antibiotic for a patient lost its allure when a U.S. company upgraded its system. Allan's firm is pinning its hopes on four cancer antibody products, now in clinical trials in Canada, as well as a combined antifungal and antibiotic.

The central concern of many observers is how long Cuba can maintain the seeming paradox of engaging in a high-risk, profit-driven industry in a state-controlled economy. "I don't think they're going to be able to do really cutting-edge biotech in a top-down world," Restifo says. "There would be hundreds of small Cuban biotech companies if the country was friendly to entrepreneurial endeavors," adds Larrick.

But Cuban researchers are optimistic. "We will succeed to sell products in the First World," predicts López-Saura. And many remain staunchly loyal to the system that made this biotech gamble. "I owe my career, my son's and daughter's careers, my master's degree, my Ph.D. degree in Sweden to the government," says CIM scientist-turned–marketing executive María Pascual López. Adds her institute's director, Agustín Lage: "These are moral values and sometimes this is difficult to explain." **–JOCELYN KAISER**

CIRCADIAN RHYTHMS

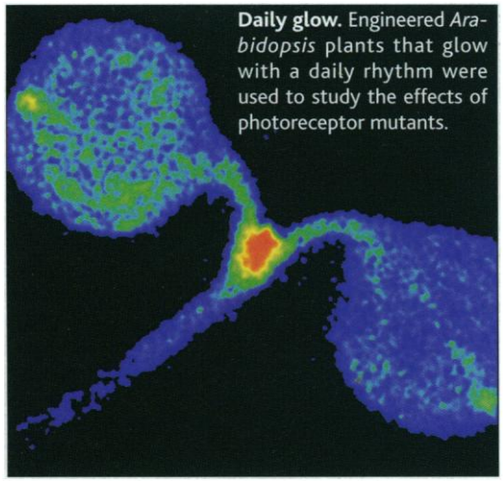
# Clock Photoreceptor Shared By Plants and Animals

**Cryptochrome, a light-absorbing molecule first discovered in plants, apparently helps light to set the daily clocks of *Arabidopsis*, fruit flies, and mice**

You arrive in a new time zone, go to a hotel, and wake up after a long, disorienting sleep. How do you tell if it's day or night? Light is a good cue, and not just for your mind. Deep in your brain, a molecular clock that oscillates with a 24-hour rhythm, pacing your physiology, also relies on light to keep it in synch with the day-night cycle. But even as researchers have discovered many of the components of the clock mechanisms that operate in organisms ranging from bacteria to humans, a major mystery has remained: the identity of the light-capturing molecules that transmit the light signal to the clock. Now, three research teams have fingered a suspect—a light-absorbing protein called cryptochrome that may play that role in organisms ranging from plants to mammals.

Last week, a team led by Steve Kay of The Scripps Research Institute in La Jolla, California, reported in *Science* that cryptochrome is a circadian photoreceptor in plants, while another paper in that same issue from Aziz Sancar's team at University of North Carolina, Chapel Hill, suggested that it might be one in mice as well (*Science*, 20 November, pp. 1488 and 1490). And this week, Jeff Hall and Michael Rosbash of Brandeis University in Waltham, Massachusetts, and their colleagues report in *Cell* that the protein plays a similar role in fruit flies.

The results indicate that cryptochrome is not the only molecule that relays light signals to the circadian clocks in these species. And the data from mice are controversial: Some researchers say that rather than proving cryptochrome is a light sensor, they suggest it could be part of the mouse clock mechanism itself.



**Daily glow.** Engineered *Arabidopsis* plants that glow with a daily rhythm were used to study the effects of photoreceptor mutants.

CREDIT: ANDREW J. MILLAR

But just finding that cryptochrome plays a role in clocks ranging cross-kingdom from plants to mammals is "an amazing development," says clock researcher Gregory Cahill of the University of Houston, and "the most extreme example of people finding homologies in clock-related genes across species." Besides filling a gap in our knowledge of circadian clocks, the work could also lead to new remedies for jet lag that might mimic or enhance the process by which light resets the clock mechanism.

That mechanism, the biological equivalent of the gears and springs in a watch, is a set of proteins whose levels rise and fall in a daily cycle. The proteins regulate their own oscillations by turning their own genes on and off; light can shift or "entrain" a clock by raising or lowering the level of a key clock protein and so influencing that feedback process. For example, in fruit flies, the clock protein Timeless (TIM) reaches high levels at night and turns its own gene off. Light causes quick destruction of TIM, allowing the *tim* gene to turn on and jump-starting the next daily cycle. To do that, the light must be captured by photoreceptors, which could be either in the same cell as the clock or some distance away, such as in neurons of the eye.

Clock researchers began to suspect that cryptochrome might be such a photoreceptor shortly after Anthony Cashmore and his colleagues at the University of Pennsylvania in Philadelphia discovered it in 1992, in the plant *Arabidopsis thaliana*. Cashmore's team showed that the protein, which is sensitive to blue light, is important for a variety of light-based growth responses in plants, such as bending toward light.

Findings such as those prompted Kay and his postdoc David Somers to test whether cryptochrome transmits light signals to the *Arabidopsis* circadian clock. To

follow the plants' rhythms, they took regulatory sequences from a gene that has a 24-hour activity cycle controlled by the clock and connected them to the gene for luciferase, an enzyme that makes a chemical that glows in the dark. They then introduced the hybrid, clock-responsive gene into *Arabidopsis* plants. By determining when the plants glowed throughout a 24-hour period, the researchers could follow how mutations in light-sensing proteins affect the clocks' responses to various light conditions.

Because light under different conditions such as twilight, midday, or deep shade is enriched in different wavelengths, Kay suspected that plants may need several photopigments to cover the spectrum and ensure that their clocks run properly in all light conditions. So he and Somers studied plants with mutations in two types of photopigments: cryptochromes, which specialize in blue light, and the phytochromes, which prefer red. They found that phytochrome mutants could not entrain their clocks to red light, while cryptochrome mutants fail to respond to blue, showing that cryptochromes and phytochromes both have roles in setting the clock.

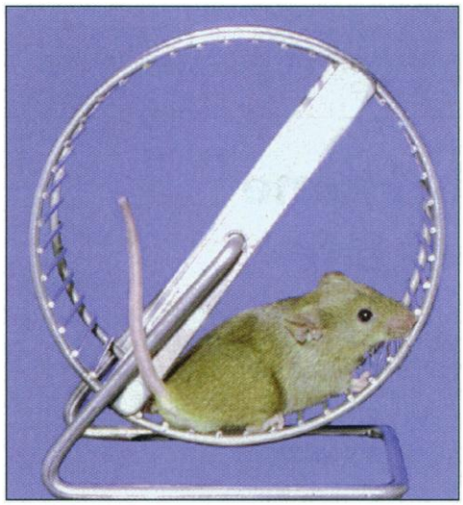
Of course, Kay points out, in natural conditions plants would never receive pure blue or pure red light, so both photopigments probably contribute to clock setting normally. But by analyzing the mutants at different wavelengths, they were able to show that both types of proteins are involved. As a result, says Houston's Cahill, "we now know more about circadian photoreception in plants than we do about most things."

Researchers haven't reached that level of understanding of clock-setting photoreceptors in animals, but in fruit flies, as in plants, cryptochrome seems to be one of at least two. That discovery came out of work begun by Ralf Stanewsky, a postdoc with Hall at Brandeis. He was searching for new mutations that affect the cycling of the clock protein PER. To do this, he adapted the luciferase assay developed in Kay's lab, in this case linking the luciferase gene to DNA sequences that control *per* gene expression. He found that one of the mutations that caused PER levels to stop cycling was in a gene that turned out to encode a fly version of cryptochrome. (Cryptochrome had been found in animals, although its function wasn't known.) Hall dubbed the gene *crybaby* (*cryb*), after a favorite Janis Joplin song.

Because cryptochrome is a light-responsive protein in plants, Stanewsky and Hall thought the clock in the fly mutants may be unable to respond to light. That idea fit with an observation made in flies by Rosbash's team at Brandeis, that light-triggered TIM degradation is most sensitive to light in the blue range—cryptochrome's specialty. So Stanewsky looked at TIM levels in the *cryb* mutant to see if they dropped in response to light. They didn't. "TIM was constantly high in light-dark cycles," Hall says. "It was not responding to light."

But cryptochrome couldn't be the fly's only circadian photoreceptor. Although the clocks were not cycling properly in most cells of the mutant flies, the flies' behavior followed a normal rhythm, and its timing could be reset by new light-dark cycles. It turned out that the clock was still cycling normally in the brain neurons that control behavioral rhythms.

That meant that the neuronal clock must be getting light signals from elsewhere, and one candidate was the photopigments in the flies' eyes, which send neuronal signals to the brain. To test this, Stanewsky crossed the *cryb* mutants with a mutant that lacks all signaling from the visual photopigments. The double mutant turned out to be "circadian blind," says Hall—the flies' behavioral cycles were unable to reset to light. That suggests that visual photopigments, as well as cryptochrome, can entrain the flies' behavioral clocks.



**Night runner.** Mice missing cryptochrome 2 are more variable in their nightly running schedule.

Cryptochrome, however, may be more than just a photoreceptor. In normal flies, the clock runs fine in the dark, with the TIM levels oscillating on their usual 24-hour schedule. If the *cryb* mutation only blocked the light response, Hall says, TIM should still follow its natural circadian cycle as though it were in the dark. But in most cells of the flies' bodies, the *cryb* mutation stops TIM from cycling at all. That means, Hall says, that *cryb* "has to be doubly defective. ... We have a strong suspicion that the CRY protein is touching the clock works, that it is interacting with the clock factors."

Sancar's data in mice suggest cryptochrome might be playing a double role in mammals, too. He and postdoc Yasuhide Miyamoto found cryptochrome in mice in two telling places: the suprachiasmatic nucleus, the brain area that is the seat of the clock in mammals, and in a layer of cells in the retina that is necessary for circadian light responses.

To see what the protein might be doing there, Randy Thresher in Sancar's lab made mutant mice that lack cryptochrome 2, one of the two cryptochromes in mice, and tested the effect on the animals' circadian clocks. In mice, light activates the production of the clock protein, PER. In the mutant mice, that activation was blunted but not eliminated. That suggests, Sancar says, that cryptochrome 2 is partially, but not wholly, responsible for transmitting light signals to the clock.

Other data support that view, he says. In behavioral tests done in Joseph Takahashi's lab at Northwestern University, the activity cycles of the mutant mice—in which they run in their wheels all night and sleep by day—at first seemed normal; they could still be changed by a shift in the light-dark cycle. But on closer examination, the timing of the activity shift was much more variable in the mutants than in normal mice, again suggesting a partial role for cryptochrome in the light-induced shifts.

What's more, mutant mice kept in the dark overreacted to light flashes. Such flashes shift the activity cycle of normal animals by about 2 hours, but in the mutant mice the cycles shifted by 8 to 12 hours. Sancar says this is consistent with what happens in some organisms when one of two light inputs to the clock are knocked out. He concludes that cryptochrome 2 is one of at least two circadian photopigments in mice, the other of which might be cryptochrome 1. His team is presently making double knockout mice to check that hypothesis.

But other clock researchers, including Russell Foster of the Imperial College of Science and Technology in London, question Sancar's conclusion. Part of their concern is due to a finding in rodents that the so-called "action spectrum," a plot of the response of the clock to different wavelengths of light, more accurately resembles the absorption spectrum of a family of photopigments called opsins than that of cryptochromes. Opsins include the photopigments the eye uses for vision, but it is clear visual opsins alone aren't responsible for clock setting. Foster's group has created mice that are totally missing all their rods and cones, the cells in the retina responsible for vision. The animals' clocks still entrain to light, says Foster, causing him to conclude that "there has to be something else" transmitting the light signals. It could be cryptochrome, he says, but adds that Sancar's data stop short of proving that.

To further test the cryptochrome hypothesis, Robert Lucas, a postdoc with Foster, suggests taking a cue from the fly experiments and crossing the rod- and coneless mice with cryptochrome mutants to see if knocking out both light-reception pathways blocks clock entrainment. Another test, says Houston's Cahill, would be to see whether the cryptochrome mutation alters the animals' action spectra for light entrainment of their clocks. If it did, he says, that would be good evidence that cryptochrome is a circadian photoreceptor.

Without such conclusive results in mice, many researchers won't accept cryptochrome as a mammalian circadian photoreceptor. Indeed, says clock researcher Carla Green of the University of Virginia in Charlottesville, Sancar's results suggest more convincingly that cryptochrome "could be part of the clock itself." That, she and others say, is the simplest explanation for overresponse to a flash of light. It could also explain the finding that the mice have altered behavioral rhythms in constant darkness, and the presence of cryptochrome in the suprachiasmatic nucleus, which governs rhythms but does not directly respond to light.

What's more, Sancar has localized cryptochrome to the cell's nucleus, where other key clock components such as PER and TIM go to regulate their own genes, a function that is at the heart of the clock's oscillating mechanism. "If I had got this data set," says Lucas, "I would be excited that maybe it has something to do with the machinery of the clock."

Indeed, what most researchers in the field find most intriguing about the new results is the suggestion that cryptochrome may have begun as a pure photoreceptor, a role it seems to maintain in plants, but during the evolution of animals may have insinuated itself into the mechanism of the clock. That would add cryptochrome to a growing list of clock proteins that evolved from photoreceptors, including a set of key clock components that are evolutionarily related to bacterial photoreceptor molecules.

One thing is for sure, says clock researcher Michael Menaker of the University of Virginia: "All of these data suggest that cryptochrome is very important. Whether it is important only as a photoreceptor, only as part of the circadian oscillator, or both, are secondary questions." For a protein discovered as a photoreceptor in plants to wind up involved in the mammalian circadian clock is quite an evolutionary leap, says Cahill: "We don't have that many evolutionary connections between plants and the mammalian nervous system."

—MARCIA BARINAGA

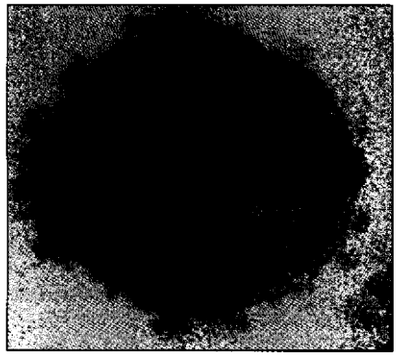
MEETING | AIDS RESEARCH

# HIV's Early Home And Inner Life

**LAUSANNE, SWITZERLAND—Until recently, Europe could boast only one major AIDS meeting: The Cent Gardes Colloquium, held biannually near Paris. This autumn, HIV researchers based in Switzerland inaugurated a second series to alternate with the Cent Gardes. The first meeting,* held here in the opulent Beau-Rivage hotel, attracted 230 researchers to discuss the latest in AIDS research from basic science to vaccine development.**

## The Core of The Matter

HIV's life cycle begins when it attacks target cells and ends when progeny viruses burst out to infect new cells. Current antiviral drugs target two enzymes involved in this cycle: reverse transcriptase, which copies HIV's RNA genome into DNA; and HIV protease, which snips viral proteins into the right sizes for assembly into mature virus particles. But some patients are resistant to these drugs or suffer side effects, and researchers are always looking for new targets to attack. A talk by biochemist Wesley Sundquist of the University of Utah, Salt Lake City, suggests that HIV's poorly understood inner core could present just such a target.



**Ripe target?** HIV's cone-shaped core might be vulnerable to new therapies.

HIV's basic structure includes an outer coat, which attaches to the membrane of a target cell, and an inner cone-shaped core, which enters the cell. This core is made up of two proteins—a large molecule called capsid and a smaller one called nucleocapsid—along with reverse transcriptase and the virus's RNA genome. The protein core appears to be a vehicle that helps transport the enzyme and the genome into the host cell. In recent years, Sundquist and his colleagues, along with other workers including Hans-Georg Krausslich's group at the Heinrich-Pette Institute in Hamburg, Germany, have shown that, under lab conditions, purified capsid spontaneously self-assembles into long, hollow tubes whose diameters roughly correspond to the varying width of HIV's conelike core.

In new work presented in Lausanne, Sundquist reported that his team was able, for the first time, to replicate cone-shaped structures similar to HIV's core by adding nucleocapsid and RNA to the capsid proteins in just the right combinations under physiological conditions similar to those in living cells. Indeed, Sundquist showed electron micrographs demonstrating that these artificial cones bear a striking resemblance to those found in actual HIV particles. "These proteins just know how to assemble in vitro," remarks retrovirologist Mario Stevenson of the University of Massachusetts Medical School in Worcester. And Didier Trono, a molecular virologist at the University of Geneva, comments that the work sheds new light on the mechanism of viral assembly, which is "really the black box of retroviral replication."

Sundquist proposed a model for how the cones might be formed from protein subunits. He showed high-resolution electron micrographs of cross sections of the hollow tubes made of pure capsid, which indicated that the tube walls are a honeycomb of hexagonal rings consisting of capsid molecules. He suggested that the addition of nucleocapsid molecules and RNA could tilt the rows of hexagons into a spiral, forcing the entire structure to narrow toward one end. As support for this model, Sundquist cited recent work by physicists Maohui Ge and Klaus Sattler of the University of Hawaii, Honolulu, who showed that fullerenes, which have a similar honeycomb structure of carbon atoms, can also be coaxed into forming conelike structures.

Stevenson and Trono think that Sundquist's experiments could lead to an in vitro assay system to test drugs rapidly for their ability to disrupt cone formation, and Stevenson suggests that the experiments might even suggest new vaccine strategies. Although previous attempts to stimulate an anti-HIV immune response using capsid proteins have largely failed, Stevenson

CREDIT: HANS GELDERBLOM/VISUALS UNLIMITED

* Colloquium of the Lémanique Center for AIDS Research, Lausanne, Switzerland, 26–28 October.