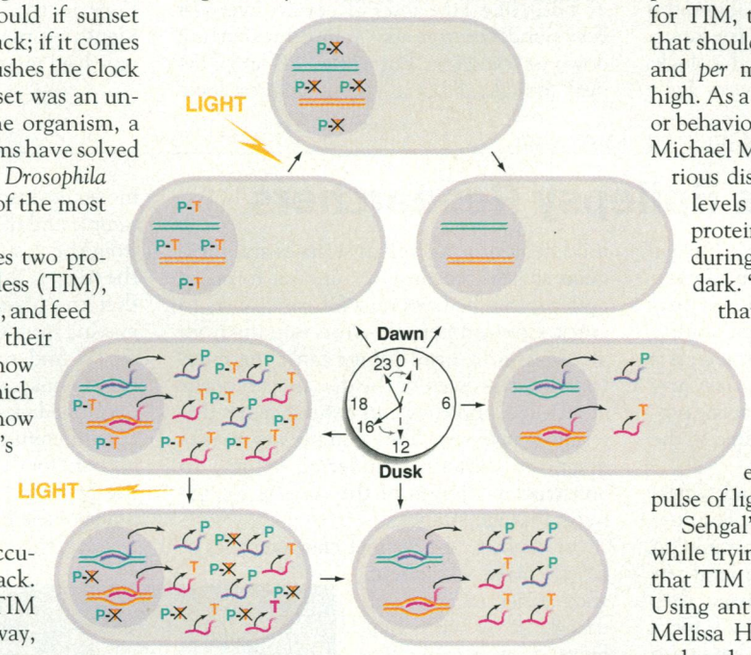
CELL BIOLOGY

Researchers Find the Reset Button for the Fruit Fly Clock

Most organisms contain molecular timekeepers known as circadian clocks, which determine their daily biological rhythms. An organism's clock is set by the light and dark cycles of day and night, and once set it will keep time even in constant darkness. But exposure to light during the dark period can reset the clock. If the light comes on early in the night—as it would if sunset were late—it sets the clock back; if it comes in the hours before dawn, it pushes the clock forward. How the clock is reset was an unsolved mystery for all but one organism, a bread mold. But now, four teams have solved the puzzle for the fruit fly *Drosophila melanogaster*, which has one of the most studied circadian clocks.

The fruit fly clock includes two proteins, period (PER) and timeless (TIM), which cycle up and down daily, and feed back on their genes to regulate their own cycling. The new results show that TIM is the reset button, which is triggered when light somehow destroys the protein in the fly's nervous system. When this happens early in the night, while PER and TIM are still being made, it delays their accumulation, setting the clock back. But later in the night when TIM and PER levels are falling anyway, it accelerates this effect, advancing the clock.



Timeshift. Light destroys TIM (T), delaying the clock earlier at night when *tim* RNA *(magenta)* is available, and advancing the clock later when TIM cannot be replaced.

Joe Takahashi, a circadian rhythm researcher at Northwestern University, describes the result as "incredibly important. This is the first specific identification of a step [directly] influenced by light within the *Drosophila* system ... and it clearly acts in a way that makes sense functionally." Not only does the work reveal how the fruit fly clock is reset, but researchers hope that comparisons with the clock of the mold, *Neurospora crassa*, will uncover unifying principles for all circadian clocks.

The four teams making the discovery were those of Amita Sehgal at the University of Pennsylvania Medical Center, which published its findings in the 8 March issue of *Cell*; Michael Rosbash at Brandeis University, which reported its results in the 14 March *Nature*; and Michael Young at Rockefeller University and Isaac Edery at Rutgers University, whose work is described on pages 1736 and 1740 of this issue of *Science*. All four came to their conclusion while trying to further hone the model of the fruit fly clock's workings.

That model began to take its present form last fall, after the *tim* gene was cloned, and TIM, like the previously characterized PER, was established as a key clock protein. Both the *tim* and *per* genes are turned on in the morning, and messenger RNAs (mRNAs) transcribed from the genes accumulate during the day. After dusk, the TIM and PER proteins somehow cooperate—perhaps by binding together and traveling to the nucleus—to stop the transcription of their own genes. As a result, first the *tim* and *per* mRNA levels, and then the protein concentrations, drop, until by morning TIM and PER are at such a low ebb that they no longer keep their genes turned off, and the genes begin making the mRNAs again (*Science*, 3 November 1995, pp. 732, 805, 808, and 811).

Even before the *tim* gene was cloned, researchers had a hint that light might impinge on this cycle through TIM. Jeffrey Price and Marie Dembinska, of the Young and Rosbash labs, found that normal flies rendered arrhythmic by keeping them in constant light appeared physiologically identical to flies lacking a functional *tim* gene. That, says Young, raised the notion that light suppresses TIM, turning normal flies into the equivalent of *tim* mutants.

Confirmation of this came via different routes in the different labs. Rosbash's and Edery's groups got their evidence after they independently confirmed the clock model's prediction that PER and TIM bind together to turn off their own genes. Their studies also showed that this TIM-PER complex breaks down at dawn, or in response to light pulses in the night. Hongkui Zeng, in Rosbash's lab, found that the breakdown seemed to be due to the destruction of TIM. "Shortly after the lights came on, TIM was gone," says Rosbash. "That suggested the light was causing TIM to go away."

Young's group arrived at the same conclusion while studying a clock mutant known as per^0, in which the flies lack a functional PER protein. Because these flies have no partner for TIM, they lose the feedback regulation that should shut down the production of *tim* and *per* mRNAs, which stay consistently high. As a result, they have no physiological or behavioral circadian rhythms. But postdoc Michael Myers in Young's group made a curious discovery: Even though *tim* mRNA levels in the mutant remain high, TIM protein concentrations dip to low levels during daylight hours, rising again after dark. "That immediately suggested to us that maybe the protein was responding to light, even in the absence of a clock," says Young. The team confirmed that hunch by putting per^0 flies in constant darkness; under those conditions TIM levels remained high, but a 1-hour pulse of light caused TIM to vanish.

Sehgal's group found the light effect while trying to verify the model's prediction that TIM enters the cell nucleus with PER. Using antibodies to TIM, graduate student Melissa Hunter-Ensor found that was true and made another intriguing finding as well: TIM disappears with the first light of dawn, while PER lingers alone. That led her to treat flies with light at various times in the middle of the night. In all cases the light caused TIM to disappear prematurely, leading her to conclude that light kills TIM.

Clock researchers knew that whatever the biochemical effect of light on the clock's protein components turned out to be, it had to explain how light can have opposing effects on the clock at different times of night. And the present findings, added to what is already known about PER and TIM, indeed provide such an explanation.

Researchers have shown that in the hours after dusk, cells have lots of *tim* and *per* mRNA cranking out TIM and PER proteins, which are just beginning to pair up but haven't yet moved en masse into the nucleus to shut down the *tim* and *per* genes. When a pulse of light comes along at this time, destruction of TIM sets back the mechanism, Young's group showed. TIM levels drop, but within a few hours they are replenished by newly made TIM, which can then pair with

PER and move into the nucleus. Consistent with this, Edery's student, Choogon Lee, found that a light pulse in the early evening delays events in the molecular clock that depend on TIM, such as PER's arrival in the nucleus and the shutting down of the *per* and *tim* genes. The effect of these changes is to set the hands of the clock back a few hours.

Later, in the hours before dawn, things are different. TIM and PER are paired up and in the nucleus, where they have shut down their genes. If light destroys TIM then, there is no mRNA around to make more TIM. And indeed, Young's group showed that light exposure at this time causes a rapid loss of TIM that is not replenished. The early demise of the TIM-PER complex allows the *tim* and *per* genes to come on a few hours earlier in the morning, advancing the clock.

Together, the findings show how light destruction of TIM can reset the clock in either direction, depending on when the light comes on. Says Takahashi: "It really is a very simple and direct mechanism."

But it's not the only way in which light can reset a clock. Last June, Jay Dunlap and his colleagues at Dartmouth Medical School reported that light resets the *Neurospora* clock not by destroying a protein, but by turning on the gene for the essential clock protein called FRQ (for frequency). But in that paper, Dunlap even predicted that light would reset the fly clock via protein suppression or destruction. The reason, Dunlap says, has to do with the time of day when the proteins are expressed.

FRQ, like TIM and PER, cycles every 24 hours and controls its cycling by shutting down its own gene. But while PER and TIM peak at night, FRQ reaches its highest level during the day. Light resets a clock by making the night situation more like day. To do that via a day protein, Dunlap says, it must increase protein levels, which is easily done by turning up the gene. In contrast, resetting a clock via a night protein requires the opposite—removal of that protein—which is just what happens with TIM. "Evolution chose two internally consistent variations on a theme, the theme being that light will act rapidly to change the level of a clock component," Dunlap concludes.

The next question for the fly researchers is how light destroys TIM. The present work, Takahashi says, will start geneticists scrambling to devise new ways to screen for mutations in that biochemical pathway, whose identity is now the latest in the long line of unsolved mysteries of the fruit fly clock.

–Marcia Barinaga

GENETIC DISEASES

Gene Perplexes Epilepsy Researchers

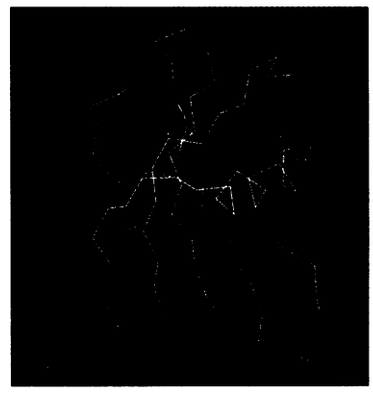
One of the benefits of using genetic linkage analysis to find the faulty gene that causes a disease is that you do not need to know anything about the function of the gene you are looking for. But the other side of the coin is that the results can be perplexing. You may finally pinpoint a gene and find that the protein it produces seems quite unrelated to the disease concerned.

That is exactly what happened to the teams led by Richard Myers of Stanford University and Anna-Elina Lehesjoki at the University of Helsinki in Finland. As they report on page 1731 of this issue, they were searching on chromosome 21 for a gene that, when mutated, causes a rare form of epilepsy known as EPM1. When they pinpointed the culprit gene, they found that it had already been described: It codes for a protein called cystatin B, which acts within cells to block the action of certain cathepsins, protease enzymes that degrade other cell proteins.

"Boy, what a surprising thing," says developmental neurobiologist Chris Walsh of Beth Israel Hospital in Boston, who is tracking down a different epilepsy gene on the X chromosome. The surprise stems from the fact that cystatin B appears to have no connection to the known mechanisms for epilepsy. Epileptic seizures occur when neurons stimulate one another to fire excessively. So the focus of epilepsy research has been on neurotransmitters and their cell-surface receptors—far from cystatin B and the regulation of protease activity inside cells.

The Stanford and Helsinki teams, however, conducted a search designed to pick up the gene at fault in EPM1 whatever its function. They chose EPM1, which causes a gradual neurodegeneration leading to mild dementia, as well as seizures and muscular spasms, because commoner forms of epilepsy can be hard nuts for geneticists to crack: Environmental factors ranging from alcoholism to head injury can contribute to them, the onset of the disease can occur at any age, and they can exhibit many different symptoms. This complexity makes diagnosis difficult, hindering efforts to construct pedigrees of the disease for genetic linkage analysis.

In contrast, the distinct characteristics of EPM1 make its inheritance easier to track, although it is very rare. Finns, however, show a higher than average incidence of many genetic diseases because they are relatively inbred from a small population dating back 2000 years. "We haven't exchanged genes with our neighbors," says Lehesjoki. The country has enough afflicted families so that the Finnish team could collect tissue samples, build pedigrees, and map the gene with a very fine resolution to a 175,000-base region of chromosome 21.



W. BODE, M. STUBBS, R. HUBER, V. TURK

Unlikely perpetrator. An absence of the protein cystatin B somehow leads to a rare form of epilepsy.

Homing in on that target region, both teams sequenced short pieces from different genes within it and compared them with known genes in sequence databases. When one of the Stanford team's sequences scored a match—with the gene for cystatin B—they went back to the pedigree samples. Using cell lines made from the white blood cells of each patient, Myers's team isolated all the RNA molecules transcribed from the DNA in each sample and then fished out just those copied from the cystatin B gene. "What really was the trigger," Myers recalls, was when "we saw that the [cystatin B] messenger RNA was missing from the patients."

To find the underlying genetic defect, Len Pennacchio, Myers's graduate student, continued sequencing the DNA. "We found two different mutations," he says, that could account for the lack of mature RNA copies of the gene and that probably lead to an absence of the protein itself.

Now the researchers are trying to work out why a lack of this protease inhibitor could lead to epilepsy. "This protein is in every cell [type] that we've seen so far" in normal individuals, says Myers, making it difficult to explain why only the brain should be affected by its absence. But Walsh sees one clue: The cathepsins that are cystatin B's targets are packaged within lysosomes, intracellular compartments resembling the synaptic vesicles that release neurotransmitters into the synapses between neurons. The resemblance leads Walsh to speculate that "a functional analogy between synaptic vesicles and lysosomes could be a very interesting avenue" for finding a link between cystatin B and epilepsy. But for now, he says he is simply "very intrigued."

–Claire O'Brien

Claire O'Brien is a writer in Cambridge, U.K.