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RESEARCH NEWS

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Experiments Illuminate Workings of Biological Clocks

By zapping fruit flies with light pulses and analyzing the biochemical consequences, investigators from the Howard Hughes Medical Institute (HHMI) at the University of Pennsylvania have pinpointed how light resets the flies' biological clock.

Since such clocks occur in humans as well as flies, these insights could suggest new ways to treat jet lag or depression, which are thought to be influenced by circadian rhythms. These types of studies may also eventually help optimize drug treatments affected by the rhythmic changes in the human body's hormone levels.

In the September 10, 1999, issue of the journal *Science*, HHMI investigator Amita Sehgal and colleagues at the University of Pennsylvania School of Medicine show how light causes cells to break down a key biological clock protein called "timeless."

Biological, or circadian, clocks operate on a roughly 24-hour cycle that governs such functions as sleeping and waking, rest and activity, fluid balance, body temperature, cardiac output, oxygen consumption and endocrine gland secretion.

In previous studies, Sehgal and others had shown that the amount of the timeless protein (TIM) present in a fly's brain is a key signal that helps to synchronize the biological to the day-night cycle. In their current work, the HHMI team was able to demonstrate the biochemical mechanism that reduces levels of TIM, a key to clock synchrony.

Light itself doesn't directly reset the clock. Rather, light strikes photoreceptors in a fly's body, which then send signals to the clock. These signals somehow reduce levels of TIM. When the level of TIM reaches a low point, the timeless gene, *TIM*, is switched on to replenish TIM's levels and reset the biological clock.

While conducting earlier studies on timeless, Sehgal hypothesized that cell structures called proteasomes played a key role in degrading TIM. Proteasomes are large agglomerations of enzymes that break down unneeded

proteins through a process called proteolysis.

To test whether proteasomes degraded TIM, Sehgal and her colleagues pulsed flies with light and found that TIM was indeed degraded in extracts prepared from cells. By contrast, extracts from flies that had not received a light pulse did not support TIM degradation.

Next, the researchers demonstrated that they could use chemicals that inhibit proteasome enzymes to block TIM breakdown. The scientists also tested the effects of such inhibitors on cultures of fly brain cells that had been either light-pulsed or not, and confirmed that the same kind of light-triggered breakdown also occurs in light-exposed cells.

For further proof of the timeless-proteasome link, the researchers checked for signs that fly cells were actually marking timeless for destruction by proteasomes. Normally, cells flag proteins for destruction and recycling by tagging them with a small protein known as ubiquitin.

Using their fly cell culture system, the scientists confirmed that TIM is indeed "ubiquitinated," demonstrating that it is a target of the proteasome. Finally, the HHMI team's experiments also revealed that degradation of TIM depended on the addition of phosphate to the protein by enzymes called tyrosine kinases.

Such basic understanding of the biological clock machinery could have clinical implications, said Sehgal, since researchers have found the clock components to be basically the same in flies and humans.

"If we understood exactly which component needed to be regulated to reset the biological clock, we could target just that component with drugs," she said. "Such drugs might aid treatment of jet lag and seasonal depression caused by absence of light in winter. It is hypothesized that other forms of depression involve clock genes. In addition, there's tentative evidence that these genes might influence whether people are naturally 'morning' people or 'evening' people.

"Finally, there are data showing that drugs such as cancer drugs may work more effectively and with fewer side effects if they were given at a specific time of day. Since so many human hormones rise and fall in circadian cycles, this effect on drug therapy seems to make perfect sense," she said.