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New Understanding of How Circadian Clocks Keep Time

Researchers have found more evidence that the sleep-wake cycle that governs the daily activities of a variety of organisms, including humans, is regulated by conserved sets of proteins.

"We are revising our understanding of how circadian rhythms are generated in humans and other species," said Joseph Takahashi, an HHMI investigator at Northwestern University. "These studies identify two new components of biological clocks that are conserved in flies, mice and possibly humans."

The research teams, which include HHMI researchers at Northwestern University and Brandeis University, say their findings are important in a number of ways: First, the work completes a first phase of the conceptual understanding of how a circadian rhythm is produced. Second, it provides evidence that the same clock genes exist across species and possibly arose in common ancestors more than 500 millions years ago.

"We now need to define the role of these clocks, which conceivably could regulate every aspect of physiology and metabolism," agrees Michael Rosbash, a Hughes researcher at Brandeis University.

Internal timing of this biological clock, which scientists call a circadian rhythm, is achieved through a simple feedback loop in which four proteins turn each other on and off. One set of two proteins works in the morning to produce the second set of molecules that accumulates during the day. In the evening, this second set of proteins inactivates the daylight-active proteins.

Although their findings complement each other, Takahashi and Rosbash studied circadian rhythms in different ways with separate research teams. Each team published their results in separate journals. "It's really gratifying that Takahashi's work complements ours so completely," said Rosbash. The fact that both teams coincidentally uncovered the clock mechanisms at roughly the same time was lucky because they mutually reinforce each team's findings, Takahashi said.

Takahashi and collaborators from Harvard University, led by Charles Weitz, and Scripps Research Institute, led by Steve Kay, published their research in two articles in the June 5, 1998, issue of the journal *Science*. Rosbash's group, which included HHMI research specialist Joan Rutila, HHMI associate Ravi

Allada and Brandeis scientist Jeffrey Hall, appeared in two articles in the May 29, 1998, issue of the journal *Cell*.

In 1997, Takahashi cloned the first known timing gene in mammals, which is called *Clock*. His latest work, in both fruit flies and mice, aimed to find the genes that interact with CLOCK to produce circadian rhythms. He also hoped to find examples of these proteins in different species.

In the first study, Takahashi and Harvard scientists searched for an "on" switch that triggers the circadian cycle. They specifically sought a protein in mice that could partner with *Clock's* protein product, designated CLOCK. They found a molecule, called BMAL1, that is produced in the same locations as CLOCK. They also found that CLOCK and BMAL1 can bind together into a "dimer," which can then turn on the mouse version of the *per* gene, *mper1*.

Then, Takahashi and his colleagues searched for a comparable "on" switch in fruit flies. Tinkering with the clockwork in fruit flies (*Drosophila melanogaster*), they found the fly version of CLOCK as well as BMAL1 and showed that they bind together as they do in mice to control the on cycle.

The researchers hypothesize that the timing loop starts when the CLOCK/BMAL1 complex activates the *per* and *tim* genes and the PER and TIM proteins increase during daylight hours. When amounts of PER and TIM reach a certain level usually by nightfall the PER and TIM proteins associate with each other and then enter the nucleus, where they inhibit the action of CLOCK/BMAL1 on their own genes. The inhibition begins to reverse the high concentration of PER and TIM that is present during the early night hours. As PER and TIM decay, they release inhibitory constraints on CLOCK/BMAL1. In the morning, CLOCK/BMAL1 is active again and promotes transcription of *per* and *tim*, and the 24-hour cycle begins anew.

Takahashi's studies were performed in normal flies and mice. Rosbash's team took a different tack in studying mutant fruit flies with aberrant circadian cycles. In their first study, they cloned the *Drosophila* version of the mammalian clock gene (which they call *clk*, and its protein CLK). In the second study, they cloned *cyc*, a gene called "cycle," that produces a protein that interacts with CLK. CYC is the *Drosophila* version of Takahashi's BMAL1. Rosbash's group also found that the CLK/CYC complex binds to and activates the *per* and *tim* genes.

Rosbash notes that while negative feedback loops such as the one that he and Takahashi are proposing are ubiquitous biological tools, most known examples, such as transcription circuits, are completed almost instantaneously. This is the first such loop that encompasses a 24-hour period.

Among the next big tasks for the researchers, said Rosbash, is to determine how the two sets of proteins interact with each other: How do PER and TIM shut off CLOCK and BMAL1? Is it through direct contact or more indirectly? Rosbash would also like to know how light influences this pathway. While it

may be possible that every cell in *Drosophila* has its own photoreceptor system, and can thus be directly stimulated by light and darkness, humans have a different regulatory system in which light perception is regulated by the eyes. How then, Rosbash asks, is circadian rhythm coordinated and synchronized within each human cell?

Takahashi is intrigued by the possibility that biological clocks may give us insight into the basis of sleep disorders and affective disorders, such as bipolar disease and depression. These disorders all involve disturbances in the sleep-wake cycle, even though that may not be the root cause, Takahashi said. Even the process of aging involves a breakdown in circadian rhythms, he said: "Increasing the efficiency of the clock won't reverse time, but it may improve a person's overall functioning."