

APRIL 26, 2007

Mutant Mouse's Internal Clock Works Overtime

Inside the bodies of animals from fruit flies to humans, internal clocks are constantly ticking, making sure activity levels and a host of physiological functions rise and fall in a 24-hour cycle. Inside cells, many of the proteins that keep the internal clocks ticking on time have their own cycles, accumulating when they are needed, then vanishing when their work is done for the day. A newly identified gene mutation in mice has now revealed how these molecular oscillations are kept on track.

Howard Hughes Medical Institute investigator Joseph Takahashi and his colleagues discovered the gene's role in regulating circadian rhythms, which they reported in the journal *Cell*, published online as an immediate early publication on April 26 and published in print on June 1, 2007. Joint lead authors in Takahashi's Northwestern University laboratory were Sandra Siepka and Seung-Hee Yoo, and another co-author, Choogon Lee, is from Florida State University. The team named the mutated gene *Overtime* because it knocks the mouse's circadian clock out of whack, lengthening its sleep-wake cycle to 26 hours.

"We have assumed that, in order for the circadian clock to cycle, these regulatory proteins have to disappear and reappear on relatively short time scales, but how that happens wasn't appreciated."

— Joseph S. Takahashi

Circadian rhythms, the activity patterns that occur on a 24-hour cycle, are important biological regulators in virtually every living creature. In humans and other animals, the brain's internal circadian clock regulates sleep and wake cycles, as well as body temperature, blood pressure, and the release of various endocrine hormones.

Since Takahashi and his colleagues discovered the first mammalian circadian rhythm gene, *Clock*, in 1997, researchers have continued to unravel the network of genes that keeps animals running on time. At least six different genes are known to be critical to the core circadian clock in mice, and these

in turn control a large, diverse group of genes with cyclical activity patterns.

Although a set of core genes has been identified that form the circadian autoregulatory feedback loop, we have had a number of reasons to suspect that there are more genes involved in the machinery, said Takahashi. These genes are likely key regulators of the central circadian machinery, and in essence adjust the timing of the ticks of the circadian clock, he said.

To identify additional genes that help fine-tune the clock's timing, the researchers screened more than 3,000 mutant mice for abnormal circadian rhythms. By tracking the activity patterns of the mice, they found that those with the *Overtime* mutation have a sleep-wake cycle of 26 hours, instead of the usual 24.

Genetic analysis narrowed down the mutation to a gene called *Fbxl3*, whose function had been previously unknown. However, other researchers had shown that *Fbxl3* belongs to a family of genes known to help trigger the destruction of certain proteins when they are no longer needed in the cell. F-box proteins are a component of the protein machinery known as the SCF ubiquitin E3 ligase complex, which tags unwanted proteins with a small molecule known as ubiquitin, targeting them for degradation.

Because a vast array of cellular functions depends on the right molecules being tagged for destruction at precisely the right time, the family of enzymes that regulates the process is vast and highly specialized. So Takahashi's team designed experiments that would show them exactly how the mutant FBXL3 protein disrupts the circadian machinery.

They established that FBXL3 is a component of the machinery that specifically targets two circadian proteins called Cryptochrome 1 and Cryptochrome 2 for degradation. The *Overtime* mutation prevents this degradation, allowing the Cryptochrome proteins to accumulate at inopportune moments. The normal disappearance of Cryptochrome proteins is necessary for key genes, called *Period*, to switch on, triggering a new cycle of the circadian clock. Thus, in the mutant mouse, the clock ticks slower, causing a 26-hour circadian cycle.

According to Takahashi, the discovery of the *Overtime* mutation is significant because it represents the first F-box protein in mammals to be shown to regulate circadian machinery. One of the surprising things is that this protein interacts so selectively with Cryptochrome proteins in the degradation process, he said. We have assumed that, in order for the circadian clock to cycle, these regulatory proteins have to disappear and reappear on relatively short time scales, but how that happens wasn't appreciated. Further studies in his laboratory will focus on the role of protein degradation in regulating the ticking of the circadian clock.