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## Early-to-Bed Mouse Illuminates Workings of Circadian Clock

Mice tend to do most of their scampering about at night, resting up during the day for the evening's activity. But fiddle with a single gene, and suddenly the animals are much livelier during daylight hours. The shift in activity in these genetically engineered mice turns out to be more than a mere nuisance to the animals' slumbering cage mates - it's helping scientists illuminate the fundamentals of biological clocks, as well as a circadian rhythm disorder that affects a small number of humans.

People with familial advanced sleep-phase syndrome (FASPS) tend to become sleepy and wake up earlier than most. They are often ready for bed around 7:30 in the evening, and ready to begin their day at 4:30 in the morning. People with FASPS—a disorder caused by alterations in a single gene—also have a shorter circadian period than those without the altered gene. Howard Hughes Medical Institute researchers have now demonstrated that mutating the same gene in mice has the same effects. A mouse model that mimics the sleep-wake patterns of human FASPS offers new opportunities to understand how biological clocks govern these cycles, as well as a wide range of physiological functions.

Howard Hughes Medical Institute investigator Louis J. Ptacek and his colleagues reported their first studies on the mutant mouse strain in an article in the January 12, 2007, issue of the journal *Cell*. Ptacek and collaborator Ying-Hui Fu are at the University of California, San Francisco; other co-authors are from the University of Utah, National University Hospital in Singapore, and Nanjing University in China.

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The mouse they developed harbors a mutant version of the human gene *Period 2* (*hPer2*), which Ptacek and his colleagues had found in earlier studies to be responsible for familial advanced sleep-phase syndrome (FASPS). When the researchers analyzed the activity patterns of the gene-altered mice, they found that the animals showed a shorter circadian period and a shift in their sleep-wake cycle equivalent to humans with FASPS.

Biological clocks operate in the brain as well as lung, liver, heart, and skeletal muscles. They work on a 24-hour, circadian (Latin for "about a day") cycle that governs not only sleeping, waking, rest, and activity, but also fluid balance, body temperature, cardiac output, oxygen consumption, and endocrine gland secretion. Researchers have also found that the circadian clock has important clinical implications, influencing, for example, the effectiveness of anti-cancer drugs.

The *hPer2* gene plays a central role in the daily cycling of the circadian clock. It is one of four clock genes that activate to produce proteins that accumulate over the circadian cycle, until they reach concentrations that shut down their own production, resetting the clock.

A central mystery, said Ptacek, has been how the single genetic mutation of the altered *hPer2* in FASPS shifts the circadian clock in humans with the disorder. In earlier work, Ptacek and his colleagues had discovered that the mutation—which causes the substitution of one amino acid for another in the protein—changes the way *hPer2* is turned on and shut off by regulatory proteins. The *hPer2* protein fails to accumulate phosphate groups, which are normally attached by enzymes called kinases to signal that a molecule should become active. However, Ptacek and his colleagues did not know the cause or consequences of this lower than normal phosphorylation.

Examining the mutant mouse allowed Ptacek and colleagues to uncover both the cause of *hPer2*'s hypophosphorylation and its consequences. They found that the single amino acid substitution in the altered protein blocks a cascade of multiple phosphorylations that would normally take place. The effect of this is to dampen the machinery that feeds back to the *hPer2* gene, decreasing production of the *hPer2* protein. It is this decreased production of *hPer2* that shifts the sleep-wake cycle.

The finding was unexpected, said Ptacek. "Based on the common model of *hPer2* function, we had a completely different prediction about what the consequences would be," he said. "We were confident that the hypophosphorylation would lead to slower degradation of the protein. Instead, we show in these experiments that normal phosphorylation of the protein is required for increased transcription of the gene."

The new finding represents only the beginning of studies using the mouse model to explore the circadian machinery, said Ptacek. Previous studies of

the circadian machinery using the fruitfly *Drosophila* or the mouse circadian machinery—while scientifically valuable—were limited by the differences between those animals' circadian systems and that of humans, he said.

“Ultimately if you really want to understand the human system, you have to study the human genes,” said Ptacek. “And there are experiments that we cannot do in humans that we can readily do in these mice that have the human gene.” Ptacek said that further studies using the mutant mouse will enable more detailed understanding of the molecules involved in the circadian machinery and the intricate feedback loops by which the circadian clock cycles.

Also, he said, the mutant mouse will enable much broader studies of the function of circadian clocks. “We know that everything about our biology—our metabolism, our cell cycle regulation and our cardiovascular, brain, lung and immune functions, are all regulated by the circadian system,” he said. “Thus, we believe that this *hPer2* mutation and other FASPS mutations might also alter other things such as immune function or predisposition to depression. Now we can extend our findings about FASPS in humans to look at these other roles of the circadian system in these mice.”