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Gene Mutation Upsets Mammalian Biological Clock

Researchers have pinpointed the cause of a genetic mutation that switches a hamster's biological clock to a 20-hour day from the normal 24-hour day.

In the April 21, 2000, issue of the journal *Science*, Joseph S. Takahashi, a Howard Hughes Medical Institute investigator at Northwestern University, and his colleagues report that they have identified the enzyme encoded by the *tau* gene, the first single-gene circadian mutation to be discovered in mammals. In 1988, researchers first described the *tau* gene in Syrian hamsters that exhibited a shorter-than-normal biological clock.

"The *tau* mutant, arguably, has been one of the most significant genetic animal models for the study of circadian rhythms in mammals," said Takahashi. Identifying the cause of the *tau* mutation offers researchers a new tool for understanding biological clocks in humans, as well as a potential target for drugs that control the biological clock.

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"With the cloning of *tau* the supply of mammalian clock mutants has been exhausted for the moment, but there is little doubt that many of the critical elements of animal clocks have been identified," writes Michael W. Young of The Rockefeller University in an editorial that appears in the same issue of *Science*.

Most biological clocks operate on a 24-hour, or circadian (Latin for "about a day"), 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 mammals, the main components of the circadian clock are found in cells in the brain. Inside these cells, the molecular components of the clock are "rewound" daily by the

effects of light and other stimuli.

Takahashi and his Northwestern colleagues, Phillip L. Lowrey, Kazuhiro Shimomura, Marina P. Antoch, and Peter Zemenides, with Shin Yamazaki and Michael Menaker at the University of Virginia, and Martin R. Ralph at the University of Toronto, used genetic and biochemical techniques to find the enzyme altered by the *tau* mutation.

"The discovery of the *tau* mutation by Ralph and Menaker more than a decade ago was extremely important because it was the first mutation shown to alter circadian rhythm in a mammal," said Takahashi. "The major problem in identifying the gene underlying this mutation was that it was found in Syrian hamsters, which were not among the model organisms addressed in the Human Genome Project." Thus, said Takahashi, genetic data and analytical techniques available for studies of mice and humans were not available for hamster studies.

To overcome these obstacles, Takahashi and his colleagues sought first to identify a wild-type Syrian hamster strain that was genetically distinct from all other Syrian hamsters in captivity. Syrian hamsters in captivity are the offspring of hamsters originally captured in 1929.

Once they found a wild-type hamster strain from a second capture made in 1971, they used a genetic subtraction method called genetically directed representational difference analysis to make detailed comparisons of the genes of the two strains of hamsters and offspring that resulted from crosses between the two strains.

Such comparisons enabled the researchers to identify specific segments of DNA associated with the *tau* locus. Using these DNA fragments, the scientists then isolated from collections of hamster genes larger DNA sequences that they compared to mouse and human genes. These comparisons showed that the hamster *tau* gene codes for CKI ϵ (casein kinase I epsilon) a type of enzyme that had never before been associated with the machinery of the mammalian circadian clock.

Interestingly enough, however, Michael W. Young and colleagues at The Rockefeller University found that the *Drosophila* circadian mutation *double-time* is also encoded by a casein kinase I that is most similar to the epsilon form of mammalian CKI.

"Our results, from both genetic linkage analysis and molecular analysis of the specific gene mutation, provide definitive evidence that CKI ϵ is a component of the mammalian circadian clock," said Takahashi.

The researchers then set out to find how the single amino acid substitutions in CKI ϵ could shorten circadian rhythm. They found that subtle structural changes introduced by the substituted amino acid altered the enzyme's ability

to function as a biochemical switch. The mutation rendered the enzyme slower at switching on proteins produced by a key circadian rhythm gene, called *PERIOD*, said Takahashi. The regular rise and fall in levels of these circadian proteins governs the length of each cycle of the biological clock. The alteration in *CKIε* effectively changes the animals' circadian rhythm from 24 to 20 hours.

According to Takahashi, the discovery of the *CKIε* gene's role in circadian rhythms offers an unprecedented opportunity for developing drugs to control the biological clock in humans.

"We now know that there are nine genes governing circadian rhythms, eight of which code for DNA transcription factors or transcriptional regulators. *CKIε* is the only gene that codes for an enzyme, which is a lot easier to use as a drug target. Such drugs could conceivably shift a person's biological clock, enabling that person to more readily adapt to changing schedules due to travel or shift work, for example."

More speculative, said Takahashi, is the idea that drugs that control circadian rhythms could be used to treat either seasonal affective disorder a depression caused by less natural light in winter or psychiatric disorders such as manic depression that seem to be associated with sleep disorders.

"The discovery of *CKIε* will also allow us to study the kinetic features of the circadian rhythm system," added Takahashi. "Right now, we can draw these nice diagrams of how the system works, but we still don't have any sense of the rates of the process. We now have *CKIε* as a target regulatory enzyme that we can study further to study what makes the clock go faster or slower in mammals."