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New Pathway to Understanding Circadian Rhythms

A gene implicated in the human nerve disorder neurofibromatosis type 1 has been shown to play an important role in a biochemical pathway that communicates crucial information about the circadian clock to various parts of the body.

Circadian rhythms, the patterns of activity 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.

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— Amita Sehgal

In an article published in the September 21, 2001, issue of the journal *Science*, Howard Hughes Medical Institute investigator Amita Sehgal and colleagues Julie A. Williams, Henry S. Su, Andre Bernards and Jeffrey Field report that the protein produced by the fruit fly version of the *neurofibromatosis-1* (*Nf1*) gene links the body's circadian machinery to other circadian-governed cells. The scientists found that the protein expressed by the *Nf1* gene appears to regulate the cellular switch, MAP kinase.

According to the researchers, the findings reveal a new facet of the circadian control system. "Very little is known about how the circadian clock communicates with the rest of the organism," said Sehgal, who is at the University of Pennsylvania School of Medicine. "We believe this work represents a significant step in understanding that communication. We now have not just a component, but an entire pathway that looks like it's mediating signals from the clock."

The scientists began to explore NF1's role in the circadian rhythm machinery because earlier evidence from other laboratories implicated a signal affected by NF1, namely, protein kinase A, in circadian rhythm pathways in flies. Subsequently, Sehgal and her colleagues were also intrigued when they heard reports from clinicians that some patients with neurofibromatosis type 1 develop sleep disorders.

Nf1 is a tumor suppressor gene, and patients with a non-functioning, mutant *Nf1* gene exhibit pale brown spots on the skin and benign tumors from abnormal nerve growth. The disorder is present in about one of every 3,000 newborns.

Sehgal obtained mutant flies with non-functioning *Nf1* from co-author Andre Bernards of the Massachusetts General Hospital Cancer Center. In charting the circadian rhythms of the mutant flies, Sehgal and her colleagues found that those rhythms were, indeed, disrupted.

"So, to make sure that this arrhythmicity was due to a lack of the *Nf1* gene, as opposed to some other factor, we did experiments to see if we could establish normal rhythms in these flies by inserting *Nf1* transgenes," said Sehgal. "And, indeed, we showed that we could restore normal rhythmicity." Additional experiments by the researchers established that the rest of the mutant flies' circadian clock machinery — governed by proteins produced by the genes *per* and *tim* — functioned normally, clearly implicating *Nf1* as a control element of the circadian clock.

The mutant flies showed other characteristics associated with neurofibromatosis, including small size and learning deficits. In flies, these deficits can be corrected, or "rescued," by switching on protein kinase A.

"We found, however, that the ability of protein kinase A to rescue the circadian rhythm abnormality was not as good as it was for the other behaviors," said Sehgal. "That suggested to us that protein kinase A was definitely not the entire story in terms of being an element of the *Nf1* pathway." Sehgal and her colleagues were aware that other research groups had shown that in mammals another cellular switch, called MAP kinase, played a role in the NF1 pathway.

"So, when we introduced mutations that affected the MAP kinase pathway, either upregulating it or downregulating it, the flies all behaved as you would expect them to if NF1 was signaling through MAP kinase," said Sehgal.

Importantly, said Sehgal, the findings by her laboratory in *Drosophila* will make the *Nf1* -mutant fly a more useful model for the study of neurofibromatosis. "Part of the reason that this work is of interest to the neurofibromatosis research community is that it validates the fly model," she said. "One of their concerns about the fly model was that the signaling pathway seemed to be different than in humans because it involved PKA rather than MAP kinase. However, we have found that for circadian rhythm effects, the signaling pathway seems to be the same.

"Thus, the work done on *Nf1* in flies, in terms of signaling pathways, might prove more relevant to our understanding of neurofibromatosis in humans, because now we know that they involve the same signaling pathway."