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Shedding Light on Circadian Rhythms

Several teams of scientists have found what may be the missing molecular link between sunlight and the circadian clocks in both mammals and fruit flies.

Circadian rhythms, the patterns of activity that occur on a 24-hour cycle, are important biological regulators in virtually every living creature. In mammals, the internal circadian clock resides in the brain, and sunlight is the cue that rewinds this clock daily. Researchers have now found a biochemical pathway that senses blue light, and thereby connects the sun to molecular components of the circadian clock, says Joseph Takahashi , an HHMI investigator at Northwestern University.

In an article published in the November 20, 1998, issue of *Science,* Takahashi and Aziz Sancar of the University of North Carolina School of Medicine in Chapel Hill report that the protein cryptochrome 2 serves as a transducer by which light drives the molecular machinery that generates circadian rhythm. Cryptochromes are light-reactive pigments found in the eye and in plants.

Cryptochrome proteins 1 and 2 (CRY1 and CRY2), which were first discovered in plants, trigger plant growth by responding to light in the blue to ultraviolet part of the spectrum. Another article in the same issue of *Science* explains how CRY1 and CRY 2 help regulate circadian rhythm in plants.

In the May 26, 1998, issue of *Proceedings of the National Academy of Sciences,* Yasuhide Miyamoto and Sancar showed that cells in the mouse and human retina express genes similar to those that code for plant cryptochromes. Takahashi, Sancar and their colleagues sought to find whether these genes were part of the eye's circadian light-sensing apparatus. There was ample circumstantial evidence: They knew that the same retinal cells that contain CRY1 and CRY2 are connected directly to the suprachiasmatic nucleus (SCN), a group of neurons deep in the mouse brain. The SCN has long been thought to be a crucial circadian pacemaker in mammals. They also knew that expression of the *Cry1* gene within the SCN follows a circadian pattern.

In the mouse retina, however, CRY2 is the predominant cryptochrome protein. To determine CRY2's role in the light-induced setting of circadian rhythms, Takahashiís and Sancarís research teams created mice that lacked the *Cry2* gene. The researchers then used these mutant mice to measure whether the absence of CRY2 affected expression of another gene, called *mPer1,* that is known to oscillate in a circadian manner.

In mice that lacked *Cry2,* light produced less than half the normal boost in *mPer1* expression within the SCN. The scientists reasoned that the residual *mPer1* expression may be caused by a second, as yet undiscovered, transducer that also conveys light signals to the SCN. The role of CRY1 in the circadian system, say the investigators, is still unclear.

Further experiments showed that eliminating *Cry2* also lengthened the circadian cycle by about one hour when the mice were kept in total darkness. This effect was not seen in wild-type mice that have *Cry2.*

This latter finding, say the investigators, suggests that CRY2 is not only the molecular link between sunlight and the circadian clock, but that it might also play a subtle role in the clock mechanism as well. "While it is clear that CRY2 represents a light transducer for the circadian system, we cannot think linearlywhen this protein is missing, so is a part of the clock itself," says Sancar.

Similar findings have also come from two new studies of cryptochrome proteins in the fruit fly *Drosophila.* In two articles published in the November 25, 1998, issue of *Cell,* HHMI investigator Michael Rosbash , Jeffrey Hall and colleagues at Brandeis University, and Stephen Kay at The Scripps Research Institute, confirm that cryptochrome is an important in bringing light into the circadian system. In addition, Rosbash says that his laboratory's work shows that without cryptochrome, "the circadian system is largely, though not entirely, dead."

Rosbash and his colleagues first characterized the fruit fly gene, *cry,* which is similar to cryptochrome genes in plants and mice. They found that the transcription of *cry* is regulated in a circadian fashion, and is closely tied to the *Drosophila* circadian system genes, *period, timeless, clock, and cycle.*

Next, Rosbash and his colleagues created strains of Drosophila with mutations that disabled the cryptochrome gene. The resulting flies lacked the normal daily oscillations in the proteins Period and Timelesss.

Despite the dramatic impact that the mutation had on circadian biochemistry, the overall circadian locomotor activity rhythms of these mutant flies was surprisingly normal. But the addition of a second mutation that leaves the fly eyes unresponsive to light created a mutant fly that exhibited no behavioral rhythms.

According to Rosbash, these studies show that the role of cryptochrome in circadian function in fruit flies "is simple and uncomplicated, and it lies at the top of the pathway." If cryptochrome does not get light, says Rosbash, the circadian system simply does not work correctly. But for certain cells there may be a second input from the eyes which apparently does not use the same cryptochrome protein for its circadian light perception.