CIRCADIAN CLOCKS

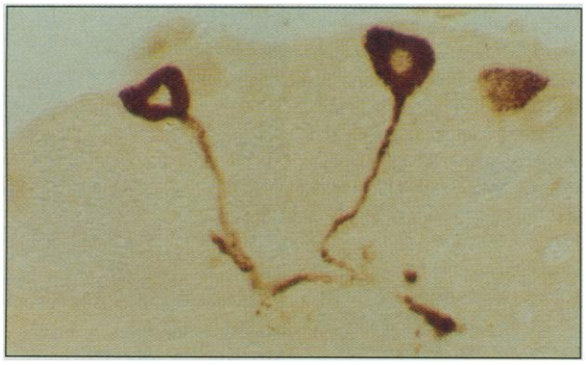
PER Protein in Silkmoths Marches to Different Drummer

It's quite a feat to take one tiny pendulum and design two completely different clocks around it. But that may be what nature has done in building the molecular clocks that control daily rhythms in two different insects. After working out the mechanism of one clock, in the fruit fly *Drosophila melanogaster*, researchers expected that their results might be widely applicable. But in this month's issue of *Neuron*, Steven Reppert and his colleagues at Harvard Medical School report that they have taken a look at the probable timekeeper in a different insect, the silkmoth *Antheraea pernyi*. The same key protein oscillator is there all right, but it seems to play a completely different role.

In the fruit fly, the protein, called PER (for period), cycles in and out of the nucleus of the fly's timekeeping neurons along with another protein, TIM (for timeless), thereby creating a 24-hour cycle by turning their own genes on and off. In the moth, however, even though PER oscillates with a 24-hour rhythm, it never enters the nucleus and appears not to be regulating its own gene. How the silkmoth rhythms are driven, and what role PER plays, remain unknown, says Reppert, "but we can say the *Drosophila* model doesn't fit these cells. There is no question about that."

Other clock researchers are still cautious about the results. They note that Reppert's team has not shown conclusively that the cells they are studying actually contain the silkmoth's clock. But the papers have "stirred the pot," says Dartmouth Medical School circadian rhythm researcher Jay Dunlap. "These papers are going to make people think much more carefully about the *Drosophila* model and whether all of its details are essential elements of the clock," adds Joe Takahashi, who studies animal clocks at Northwestern University. Indeed, the papers are likely to spur a new wave of investigation of the mechanisms of other insect clocks.

Reppert's team didn't set out to shake up the field—just to see how universal the fruit fly clock mechanism is. Based on work from several labs, this mechanism involves two key genes, *per* and *timeless* (*tim*), that become active in the early morning, causing the levels of their protein products, PER and TIM, to rise in the cytoplasm of the fly's clock neurons. The proteins pair up and travel together into the nucleus, where they begin to accumulate in the early evening, eventually shutting off their own genes. During the night, with the genes off, PER and TIM protein levels drop until they can no longer keep the genes suppressed; by morning the genes turn on and the cycle begins again.



Doughnuts. Neurons in the silkmoth brain show brown PER staining in their cytoplasm, but the nuclei are unstained.

SAUMAN AND REPPERT, *NEURON* 17(5), PP. 889–900 (1996).

To see whether the same thing happens in the silkmoth, an insect chosen because it has several well-studied circadian rhythms that govern such behaviors as flight and pheromone release, Reppert's group cloned the moth's *per* gene. Then postdoc Ivo Sauman used antibodies made to the gene's protein product to search the silkmoth brain for neurons making PER. In a part of the brain already shown by Jim Truman, of the University of Washington, to contain the silkmoth clock, the antibodies stained eight neurons—apparently the cells making up the clock.

He and his colleagues had reason to expect that the silkmoth clock would work the same way as the fruit fly's. For one thing, they had inserted the silkmoth *per* gene into fruit flies and found that its protein functions just like fruit fly PER. Also, they found, PER seems to be part of a clock in the silkmoth's eye: There, as in flies, it cycles in and out of the nucleus.

But much to their surprise, PER behaves differently in the neurons of the silkmoth brain. The first sign of that difference came when the PER antibody stain made the neurons "look like doughnuts," says Reppert, because all the PER protein was in the cytoplasm. Indeed, although PER levels cycle up and down during the 24-hour day, Sauman found no evidence that PER ever enters the nucleus. "It is as if there are two systems of PER regulation in the silkmoth," says Reppert, "one system in the eye, which looks and smells like *Drosophila*, and one in the brain, which seems to be totally different."

If PER isn't going to the nucleus to shut its gene off in the brain neurons, what is driving its cycling there? Reppert has a tantalizing, if unorthodox, suggestion. He and Sauman found a so-called "antisense" *per* RNA in the neurons, transcribed from the DNA strand of the *per* gene complementary to the one that makes the mRNA coding for the PER protein. Intriguingly, the antisense RNA level cycles, reaching its peak just when the *per* mRNA and PER protein are at their lowest. Because antisense RNAs can block expression of their corresponding genes, Reppert suggests that this antisense RNA could be driving the oscillation of PER mRNA and protein levels.

But before accepting that the silkmoth clock could be so different from the one in flies, other researchers want to see better evidence that the eight neurons are indeed the silkmoth clock, and that they are driven by PER. Jeff Hall, who studies circadian rhythms at Brandeis University in Waltham, Massachusetts, notes, for example, that PER needn't always drive a clock because it is present in some nonneuronal fruit fly cells where it has no clock function. He also points out that while the Reppert team showed that PER is essential for the circadian clock in silkmoth embryos, they haven't shown the same for adults.

Mike Young, of Rockefeller University in New York City, suggests that instead of running a clock, the PER in the silkmoth neurons may be part of a so-called "slave oscillator" whose cycles are driven by a clock located elsewhere. Reppert plans to check that by removing the neurons from moth brains, and asking whether their activity and PER levels still oscillate when the cells are isolated from other influences.

In spite of these uncertainties, as yet unpublished evidence from Bambos Kyriacou of the University of Leicester, U.K., Kathy Siwicki of Swarthmore College, and their colleagues supports Reppert's finding. These researchers looked at PER distribution in the brains of houseflies, which are close relatives of fruit flies, focusing on neurons that are the direct counterparts of the fruit fly pacemaker cells. In those neurons, they found, PER never enters the nucleus.

That means Reppert's finding is "not an anomaly," says Kyriacou. Indeed, he points out that no one has shown that PER cycles in and out of the nucleus in any species besides *D. melanogaster*. "It could be that *melanogaster* is unusual," he proposes. Kyriacou has begun to look at PER cycling in other *Drosophila* species and expects that he might find variations even in these close relatives of *melanogaster*. "Bigger surprises are yet to come on PER," he predicts.

–Marcia Barinaga