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Morning 'Alarm Clock' Resets Evening Clock

Scientists have found that the biological clock that sparks a burst of morning activity in fruit flies plays extra duty in resetting a second clock, which jumpstarts activity in the evening. They speculate that resetting the clocks daily ensures that the two clocks remain in synchrony, so the fly can maintain a regular, 24-hour cycle.

The research team led by Michael Rosbash, a Howard Hughes Medical Institute investigator at Brandeis University, reported its findings in the November 10, 2005, issue of the journal *Nature*. Rosbash collaborated on the studies with lab members, Dan Stoleru, Ying Peng, and Pipat Nawathean.

Biological clocks in both flies and humans operate on a 24-hour, or circadian, cycle. In humans, the clock governs sleeping and waking, fluid balance, body temperature, cardiac output, and oxygen consumption. In the fruit fly *Drosophila*, however, the circadian clock has its most pronounced effects on the fly's level of activity. In both flies and humans, the clocks are circuits composed of neurons that naturally oscillate with a circadian periodicity. Inside these cells, the molecular components of the clock are "rewound" daily by the effects of light and other stimuli.

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- Michael Rosbash

In earlier studies, Rosbash and his colleagues discovered the separate morning and evening circadian clocks in *Drosophila*. However, he said "between nothing and almost nothing" was known about how these two clocks were synchronized to maintain their 24-hour periodicity. "There has been a suspicion that there were synchronizing mechanisms in mammals as

well as flies, because when the key regulatory neuropeptides are eliminated that synchrony goes awry,” Rosbash said. “It’s as if the clock cells have lost the capacity to track what their counterparts are doing.”

In their studies, the researchers genetically manipulated the speed of either the morning clock circuit (M-cells) or the evening circuit (E-cells) to explore how they affected one another. “These experiments enabled us to distinguish whether the mechanism was ‘fastest wins,’ or whether one set of cells is dominant,” said Rosbash. “Our experiments revealed that the latter model is the case—that the morning cells reset the evening cells to maintain synchrony.” Their studies showed that the morning cells reset the evening cells with a daily signal. Further investigation revealed that the evening cells run at a genetically programmed pace between resetting.

To their surprise, however, the studies of the neuronal clock circuitry uncovered yet another distinct circadian clock circuit that not only escapes resetting but has no apparent effect on the flies’ activity. This circuit involves a pair of cells called DN2s. “This circuit just fell out of the data, and we hadn’t imagined it even existed,” said Rosbash. “We really have no clue what its function is.” Rosbash speculated that since the DN2s first appear in fly larvae—persisting through metamorphosis into the adult - the circuit may provide circadian regulation of some physiological function particularly important to larvae.

In further studies, the researchers are exploring the molecular basis of the synchronizing machinery. “We’d like to map the circuitry between the two sets of cells and also figure out what happens inside the evening cells to reset them,” he said. “And even more intriguing, we have evidence that there are reciprocal signals between the two circuits, in which the E-cells send signals back to the M-cells. We really haven’t a clue how these work.”

Overall, this two-way control of circadian period has probably evolved because it is so critical to survival, said Rosbash. Not only is the synchrony important for maintaining a precise 24-hour cycle but also for measuring changes in day length with the seasons. “We speculate that these oscillators signal to one another in part because day-length measurement in animals—including insects and mammals—tells the animal when to undergo seasonal changes,” he said. For example, day length governs the ability of animals to give birth only in the spring, when conditions for survival are optimal.

While much less is known about the details of the circadian system in mammals than in fruit flies, the principles are likely to be the same in both. “The mammalian circuitry is more like a salt-and-pepper mix of cells, unlike in the fly, in which the cells are better segregated,” he said. “This makes the mammalian system more difficult to manipulate experimentally, but we believe that the two systems function broadly the same way.”