New research helps to explain how temperature shifts the circadian clock

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As part of studies of the circadian clock, Kidd and others in the lab put fruit flies in glass tubes to monitor their daily activity. The molecular basis for the flies' clock is well understood, but the reason its 24-hour period does not lengthen or shorten with temperature is a long-standing mystery. Credit: Zach Veilleux/The Rockefeller University

For many living things, a roughly 24-hour internal clock governs the rhythms of life—everything from sleep in animals, to leaf opening in plants and reproduction in bread mold. Scientists have come to understand much about this internal time-keeping system, but one important aspect, its complex response to temperature, remains enigmatic.

The reason is that while warming and cooling cause the clock to shift forward or backward, they cannot shorten or lengthen its 24-hour cycle. New research, published Nov. 2, 2015 in the *Proceedings of the National Academy of Sciences*, explores how this is possible.

"Our research suggests an explanation: The internal gears within the clock—the cyclical activity of genes and concentrations of proteins—do not change with temperature, so the length of the cycle stays the same," says senior author Eric Siggia, Viola Ward Brinning and Elbert Calhoun Brinning Professor at Rockefeller's Center for Studies in Physics and Biology.

"Meanwhile, the core mechanisms of the clock appear to be linked to external pathways that are sensitive to temperature. This external coupling can cue the clock to skip ahead or backward," Siggia says.

This study is the result of a collaboration between Siggia's lab and Michael W. Young's Laboratory of Genetics. It builds upon growing evidence calling into question a model that has, for decades, been used to explain temperature-induced shifts.

A new explanation

In recent decades, researchers have uncovered the genetic processes that drive the clocks of many different species. In fruit flies, for example, a pair of proteins called Period and Timeless are transcribed from their genes, and after a series of steps, eventually repress their own production. This cycle takes approximately 24 hours, regardless of the temperature at which an organism lives. This makes the circadian clock an oddity, since temperature alters the rate at which most biological processes take place.

For decades, the dominant explanation for 24-hour period's independence from temperature, a phenomenon known as temperature compensation, relied on the heat-sensitivity of the chemical reactions that run the time-keeping cycle. According to this theory, the effects of temperature on the many chemical reactions in the circadian cycle cancel each other out, leaving the period unaffected by changes in temperature.

But increasingly, studies have suggested something else is going on. For example, a 2010 study showed that in fruit flies whose protective heat and stress response has been inhibited, the clock hardly shifts with temperature changes. Since the conventional model predicts that everything is temperature sensitive, no single pathway should be this influential.

Previous work in Siggia's lab suggested an alternative explanation. The investigators used computational tools to simulate the evolution of gene networks, including a model of the circadian clock. Their findings suggested that temperature-induced shifts of the clock involve genetic pathways separate from the molecular cycles at the core of the clock, but linked to them, and that the core clock is actually insensitive to temperature.

Proportional at different temperatures

The recent study's lead author, Philip Kidd, a postdoc in the labs of Siggia and Young, set out to test this hypothesis. Using a conceptual model and molecular experiments in flies, he examined how the oscillations of individual components of the clock, such as concentrations of the protein Timeless, changed with temperature.

"If our prediction is correct, and the core mechanism of the clock does not respond to temperature, then the fluctuations in a particular component at different temperatures should remain proportional to one another—that is, we should be able to rescale them so they align," Kidd says. "And when we examined the transcription rate of Timeless, as well as its subsequent levels at three temperatures, that was precisely what we found."

Meanwhile, in a fly with a mutation that allows the length of the clock's period to change with temperature, the curves describing fluctuations in Timeless at the three temperatures shifted around and changed shapes, and were no longer proportional, confirming the premise of Siggia's earlier model.

"The mechanism by which the clock can partially ignore temperature has remained a key problem for those of us seeking to understand how it functions. Our work, and that of others, points to a general system that could explain this mystery and lead to a comprehensive understanding of these clocks," says Young, a study co-author and the Richard and Jeanne Fisher Professor.

More information: Philip B. Kidd et al. Temperature compensation and temperature sensation in the circadian clock, *Proceedings of the National Academy of Sciences* (2015). DOI: 10.1073/pnas.1511215112

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