Study reveals key molecular interaction that sets the timing of our biological clocks

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Molecular interactions drive the biological clocks in our cells, synchronizing our bodies with the 24-hour cycle of night and day. Certain mutations can shorten clock timing, making some people extreme "morning larks" because their internal clocks operate on a 20-hour cycle. Credit: Jonathan Philpott via Bing Image Creator

Molecular clocks in our cells synchronize our bodies with the cycle of night and day, cue us for sleep and waking, and drive daily cycles in virtually every aspect of our physiology. Scientists studying the molecular mechanisms of our biological clocks have now identified a key event that controls the timing of the clock.

The new findings, published May 18 in *Molecular Cell*, reveal important details of the molecular interactions that are disrupted in people with an inherited sleep disorder called Familial Advanced Sleep Phase Syndrome (FASP). The syndrome is caused by a genetic mutation that shortens the timing of the clock, making people extreme "morning larks" because their internal clocks operate on a 20-hour cycle instead of being synchronized with the 24-hour cycle of our planet.

"It's like having permanent jet lag, because their internal clock never gets caught up with the daylength," said corresponding author Carrie Partch, professor of chemistry and biochemistry at UC Santa Cruz. "The FASP mutation was discovered 20 years ago, and we knew it had a huge effect, but we didn't know how or why."

The FASP mutation affects one of the core clock proteins, called Period, changing a single amino acid in the protein's structure. The new study shows how this one change disrupts the Period protein's interactions with a kinase enzyme (casein kinase 1), decreasing the stability of the Period protein and shortening an important step in the clock cycle.

First author Jonathan Philpott, a postdoctoral researcher in Partch's lab at UCSC, explained that the kinase enzyme regulates Period by attaching phosphate groups (a process called phosphorylation), and there are two different parts of the protein where it can do this. Phosphorylation of the "degron" region tags the Period protein for degradation, whereas phosphorylation of the FASP region stabilizes it. The balance between degradation and stabilization determines the length of the clock cycle, and the FASP mutation tilts the balance toward degradation of Period and shortening of the cycle.

"There's about a four-hour shortening of the clock when you have this FASP mutation," Philpott said.

An important finding of the new study is that the phosphorylated FASP region inhibits the activity of the kinase. This feedback inhibition mechanism enables Period to effectively regulate its own regulator, slowing the phosphorylation of the degron region and lengthening the cycle. "We need this pause button to slow down what would otherwise be very fast biochemistry," Partch said.

The researchers showed that the inhibition results from binding of the phosphorylated FASP region to a particular site on the kinase, which could potentially be targeted by a drug.

"We can start thinking about this as a tunable system," Philpott said. "We've identified regions on the kinase that are potentially targetable to tune its activity for therapeutic applications."

Partch noted that most drugs that target kinases work by blocking the active site of the enzyme. "That's basically a hammer that turns off the kinase activity," she said. "But with the discovery of new pockets unique to this kinase, we can target those to modulate its activity in a more controlled way."

This could help not only people with Familial Advanced Sleep Phase Syndrome, but also people whose sleep cycles are disrupted by shift work, jet lag, and other challenges of the modern world.

Another striking finding in the new study is that the feedback inhibition of the kinase enzyme by the Period protein also occurs in fruit flies, even though the phosphorylation sites are different.

"It turns out the short-cycle mutant in Drosophila, discovered in 1970, does the same thing as the short-cycle FASP mutation in humans," Partch said. "This mechanism has likely been in place throughout the evolution of multicellular animals. The fact that it's been rooted in place for such a long time suggest it's fundamental to making biological clocks on Earth have a 24-hour cycle."

Partch and Philpott said their collaborations with multiple labs at other institutions enabled them to go beyond their experimental observations to study the clock mechanisms from a variety of angles. The study included the use of NMR spectroscopy, simulations of molecular dynamics, and genetically engineered human cell lines, as well as characterization of the same molecular mechanisms in humans and Drosophila fruit flies. "It was a terrific collaborative team," Partch said.

More information: Carrie L. Partch, PERIOD phosphorylation leads to feedback inhibition of CK1 activity to control circadian period, *Molecular Cell* (2023). DOI: 10.1016/j.molcel.2023.04.019. www.cell.com/molecular-cell/fu … 1097-2765(23)00290-3

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