A snooze button for the circadian clock

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We may use the snooze button to fine-tune our sleep cycles, but our cells have a far more meticulous and refined system. Humans, and most other organisms, have 24-hour rhythms that are regulated by a precise molecular clock that ticks inside every cell. After decades of study, researchers are still identifying all the gears involved in running this "circadian" clock and are working to put each of the molecular cogs in its place.

A new study by Rockefeller University scientists now shows how two of the key molecules interact to regulate the clock's cycle and uncovers how that switch can go haywire, identifying one potential cause of heritable sleep disorders.

In research published in *PLoS Biology*, Michael Young, Richard and Jeanne Fisher Professor and head of the Laboratory of Genetics, and his colleagues show that the circadian clock contains the equivalent of an on/off switch that's controlled by an enzyme called doubletime. Doubletime, or DBT, was originally named for the way that mutations of the gene create flies with a fast-running clock. DBT works by attaching phosphate groups to proteins in a process called phosphorylation.

And the protein it phosphorylates, called period or PER, plays a substantial role in the timing of the clock itself, regulating the activity of other genes as it cycles on and off with a 24-hour rhythm. Researchers knew DBT played a role in regulating the period protein by attaching phosphates to it. But Young's new study shows that DBT can either suppress or activate PER, by placing phosphates at different sites.

With the discovery that DBT has not one but two separate phosphorylation targets on PER came the realization that the enzyme is acting essentially as a switch. “It’s a phosphorylation switch controlled by doubletime that determines whether the protein is active at all,” Young says. During the “off” phase, the cell churns out PER proteins that are stable but inactive, kept so by the presence of phosphate groups at that first target site. During the “on” phase, the phosphate group in the second target site activates the protein but destabilizes it so that PER is only active for a few hours. After that, the cell begins accumulating inactive protein again and the cycle begins anew.

Young and his colleagues also uncovered mutant flies with a DBT-dependent, accelerated clock: Their period proteins were missing the first target phosphorylation site that should suppress phosphorylation of their “on” switch. As a result, their period proteins never completely stabilized. “If you can’t phosphorylate the first site, you automatically skip to the second site, phosphorylate it prematurely and produce a hyperactive repressor,” Young says. “With a repressor that acts too soon and goes away too quickly, you get a short-period phenotype.” In other words, you get a fly that wakes up too soon and falls asleep too early, a fly with a fast-running clock.

Scientists have been studying human families that have members who appear to suffer from a heritable version of this short-period phenotype, termed FASPS (for Familial Advanced Sleep Phase Syndrome); these people wake up before dawn and crash before sunset. Studies of one of these families in Utah has shown a similar period protein phosphorylation defect.

Now, the researchers believe that effects like those revealed by Young’s group in the fly could very well be what’s causing the fast-running clocks in people afflicted with FASPS. “Many of the features that they’re seeing in humans are consistent with what we’re finding in flies,” Young

says. “So it may help us understand the human syndrome as well.”

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