Researchers better understand biological clock

October 12 2007

Researchers at Harvard University and the Howard Hughes Medical Institute (HHMI) have discovered that a simple circadian clock found in some bacteria operates by the rhythmic addition and subtraction of phosphate groups at two key locations on a single protein. This phosphate pattern is influenced by two other proteins, driving phosphorylation to oscillate according to a remarkably accurate 24-hour cycle.

Writing this week in the journal *Science*, the scientists describe what causes a trio of proteins, if placed in a test tube with the common biochemical fuel ATP as a source of phosphate, to function as a minimalist biological clock of sorts, maintaining an accurate circadian rhythm for long periods of time.

The new Harvard work builds upon research reported in 2005 by biologist Takao Kondo and colleagues at Nagoya University in Japan. That team initially reported that a circadian clock could be reconstituted in a test tube solely with three proteins and ATP.

“The most striking feature of this circadian oscillator is its precision,” says Erin K. O’Shea, professor of molecular and cellular biology and chemistry and chemical biology in Harvard’s Faculty of Arts and Sciences (FAS), director of the FAS Center for Systems Biology, and Howard Hughes Medical Institute investigator. “Even in the absence of external cues — in total darkness — these minuscule protein-based clocks can maintain precision to a small fraction of a day over several

weeks."

O'Shea, postdoctoral researcher Michael J. Rust, graduate student Joseph S. Markson, and colleagues studied circadian rhythms in cyanobacteria, better known as blue-green algae. These simple organisms, responsible for some 70 percent of the Earth's photosynthesis, devote most of their energies toward just two biological processes: photosynthesis and reproduction.

The scientists scrutinized the activity of three bacterial proteins known as KaiA, KaiB, and KaiC. They found that during the daytime, KaiC is cyclically phosphorylated at two amino acid residues: first at a specific threonine, and then at a specific serine. During nighttime hours, the two amino acids are dephosphorylated in the same order.

The KaiA protein promotes the phosphorylation of KaiC, and KaiB, sensing one of the phosphorylated forms of KaiC, blocks KaiA's activity, creating an intricate biochemical dance that results in a nearly perfect 24-hour oscillation. The researchers' subsequent mathematical analysis confirmed that this distinctive dynamic would, in fact, reproduce a circadian period.

The bacterial proteins studied by O'Shea, Rust, Markson, and colleagues are not known to exist in humans, but the researchers say their findings illuminate general feedback mechanisms that could serve to establish chronological oscillations in a whole host of organisms.

"It's unknown whether such a mechanism is at the core of all circadian clocks," says Rust, a postdoctoral researcher in Harvard's Department of Molecular and Cellular Biology. "It's the simplest chemical oscillator known, and we are looking at it as a possible model for other species."

O'Shea says the 2005 finding by Kondo and colleagues that a

cyanobacterial circadian clock could be recreated in a test tube using only three proteins and ATP surprised researchers because it showed that some circadian rhythms are driven solely by protein-protein interactions.

“It demonstrated that circadian clocks can operate independently of DNA and most cellular components, contradicting the previous prevailing theory that an entire organism was likely needed to maintain a clock,” she says.

O’Shea, Rust, and Markson’s co-authors are William S. Lane at Harvard and Daniel S. Fisher at Stanford University. The research was sponsored by HHMI and the National Science Foundation.

Source: Harvard University