

OCTOBER 04, 2007

Learning What Makes a Simple Circadian Clock Tick

Researchers have figured out how a bacterial circadian clock ticks away using only three interacting proteins. They have shown that the cellular equivalent of this clock's pendulum and timing gear is so rugged that it can keep precise time for weeks even after its components have been isolated from bacteria and placed in a test tube.

The researchers still don't know whether the bacterial clock has anything to do with the 24-hour circadian clock that governs the physiology of plants and animals, including humans. The ticking of that circadian clock—which regulates such functions as sleeping and waking, rest and activity and endocrine gland secretion—is based on the periodic cycling of gene activity.

"Now that we understand this core timekeeper, we want to learn how it receives information from the environment in the form of light."

— Erin K. O'Shea

Howard Hughes Medical Institute investigator Erin O'Shea, her postdoctoral fellow Michael J. Rust and her graduate student Joseph S. Markson at Harvard University published their findings October 4, 2007, in *Science Express*, which provides electronic publication of selected *Science* papers ahead of print.

In their studies, the researchers sought to understand how the three proteins of a circadian clock found in cyanobacteria, also known as blue-green algae, produce steady rhythmic oscillation of phosphorylation. In earlier studies, Takao Kondo and colleagues at Nagoya University in Japan had discovered that the oscillator does not depend on the rhythmic cycling of genes, as do other circadian clocks. It is thought that circadian clocks allow cells in cyanobacteria to anticipate daylight, during which time cells carry out photosynthesis.

It has been the dogma in the field that circadian clocks are transcriptional-translational oscillators—in which a gene produces a protein that then eventually feeds back to turn off its own production, said O'Shea. That cycle of production and repression continues with a 24-hour periodicity.

In fact, the same is true of the circadian clock in the cyanobacteria. But the remarkable thing about the clock in cyanobacteria—and it was an enormous surprise to the research community—was the Kondo lab's discovery that its core timekeeping mechanism consists only of three proteins.

Rather than relying on the rhythmic activity of genes, this timekeeping mechanism is post-translational, meaning that it depends on biochemical interactions of the three proteins to keep time, said O'Shea. Thus, the clock can tick along for weeks with only its three proteins in a test tube powered by adenosine triphosphate as its sole source of energy, she said.

The clock in blue-green algae is somehow layered onto the gene-controlled circadian clock, said O'Shea. And like the venerable Timex watch that takes a licking and keeps on ticking, it is sturdy enough to persist inside the molecular chaos of cells and through countless rounds of cell division.

The centerpiece of the clock is a protein called KaiC, an intricate enzymatic machine whose molecular ticking is caused by the regular addition and subtraction of phosphate molecules at two specific sites on the protein. The KaiC protein is self-sufficient, in that it can phosphorylate and dephosphorylate its two sites on its own. However, KaiC is regulated by two partners in the ticking triumvirate—KaiA and KaiB.

While Kondo and his colleagues had established that the clock in cyanobacteria is comprised of only three proteins, the mechanistic details of how those proteins cycled to keep time remained unknown, said O'Shea. So, she and her colleagues set out to creatively tinker with the proteins to see if they could understand the nature of the cycling mechanism.

In their experiments, the researchers teased apart the steps in the oscillation process by selectively mixing the proteins at different stages of KaiC phosphorylation and observing how the oscillation proceeded. This approach permitted the researchers to model the partial reactions of the phosphorylated proteins.

These experiments revealed how KaiA and KaiB enhanced or inhibited the addition or deletion of phosphate molecules in KaiC. And their analyses showed how the interaction with KaiA and KaiB leads to the molecular ticking—with KaiC cycling through four distinct states—totally unphosphorylated, only phosphorylated on one or the other of its two sites, and totally phosphorylated.

Something really amazing came from our studies—that the time-scale of these reaction rates is extremely slow, which is where the twenty-four-hour period comes from, said O'Shea.

The researchers used insights from their biochemical experiments to create a simple mathematical model of the interactions of the three proteins. Just by including in our model the partial reactions, and not the full oscillating reaction, we were able to recapitulate the sustained oscillation, said O'Shea. The model could predict the essential features of the oscillator—its period,

amplitude of phosphorylation and sequential appearance of the phosphorylation states. This output was particularly remarkable because the model was so simple—only three equations—whereas other models of this system have been quite complicated, she said.

In future studies, O'Shea and her colleagues hope to extend their understanding of the clock in cyanobacteria. Now that we understand this core timekeeper, we want to learn how it receives information from the environment in the form of light, she said. Our goal will be to recapitulate this control mechanism in the test tube to understand it at a mechanistic biochemical level, as we now understand the clock itself.