Circadian rhythms studies reveal new temperature regulator and track clock protein across a day

May 15 2009

Dartmouth Medical School geneticists have made new inroads into understanding the regulatory circuitry of the biological clock that synchronizes the ebb and flow of daily activities, according to two studies published May 15.

Research on the relationship between clocks and temperature, reported in *Cell*, offers insight into a longstanding puzzle of temperature compensation: why the 24-hour circadian rhythm does not change with temperature when metabolism is so affected.

A related study, in *Molecular Cell*, tracks a clock protein in action, mapping hundreds of highly choreographed modifications and interactions to provide the first complete view of regulation across a day.

The new work adds clarity to the molecular underpinnings of circadian clocks, the finely tuned cellular timekeepers that drive most organisms. Circadian systems are biological oscillators that orchestrate activities through an elaborate network of interactive proteins and feedback loops. All clocks rely on transfer of phosphate groups, called phosphorylation, to clock proteins for setting the 24-hour cycle.

Both studies looked at phosphorylation of the frequency (FRQ) clock protein, a central feedback cog in the fungal clock system. They build on the research of team leaders, Drs. Jay Dunlap and Jennifer Loros, who

have documented the workings of FRQ and most other components in the Neurospora clock.

"The Cell paper describes how the cell uses phosphorylation of a clock protein to keep the period length of the cycle close to the same across a range of temperatures. This phenomenon, called temperature compensation, is one of the few canonical properties of rhythms that still lack molecular description," said Dunlap.

"The one in *Molecular Cell* describes collaborative work with Dr. Scott Gerber in the Norris Cotton Cancer Center. We used mass spectrometry to follow the degree of phosphorylation of over 75 sites on the FRQ clock protein across the day. Most proteins have one or a few phosphorylations, so following these across time is a major technical achievement as well as being informative for the clock biology."

In *Cell*, the researchers suggest a new role for the clock-associated enzyme, casein kinase (CK)2 as a key control for temperature compensation. Pursuing two uncharacterized circadian protein mutants shown to affect compensation in an unusual way, the investigators identified different subunits of the same enzyme, CK2.

They developed new ways to manipulate the genome and showed, by controlling expression, that the level of CK2 dictates the form of compensation through the phosphorylation of the clock protein FRQ. The property is unique to CK2 and shared with none of the other similar enzymes implicated in clock function.

Coauthors in addition to Dunlap, professor of genetics and Loros, professor of biochemistry and of genetics, are Arun Mehra, Mi Shi, Christopher L. Baker, Hildur V. Colot.

The second study traced protein interactions throughout the cycles to

demonstrate how phosphorylation controls circadian rhythm. Using a heavy isotope labeling method and quantitative mass spectrometry, the researchers pinpointed a near record number of modifications on FRQ and described how each appears and disappears over the day.

Moreover, their methods facilitated the identification of interacting proteins to track and correlate changes in the core circadian network. They determined the clusters and locations of known sites, and through mutational analysis identified novel functional domains to create a dynamic view of a clock protein in action.

Source: Dartmouth College