Research reveals how cells tell time

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The fuzzy pale mold that lines the glass tubes in Dr. Yi Liu's lab doesn't look much like a clock. But this fungus has an internal, cell-based timekeeper nearly as sophisticated as a human's, allowing UT Southwestern Medical Center physiologists to study easily the biochemistry and genetics of body clocks, or circadian rhythms.

In a new study appearing online this week in the *Proceedings of the National Academy of Sciences,* Dr. Liu and his co-workers have found that this mold, which uses a protein called FRQ as the main gear of its clock, marks time by a sequence of changes in the protein's chemical structure.

Dr. Liu said the new finding might someday help researchers develop treatments for human sleep disorders and other problems associated with a faulty biological clock.

"This timekeeping protein is really the core component of the circadian clock," said Dr. Liu, professor of physiology at UT Southwestern and senior author of the study.

Despite the evolutionary distance from mold to man, mechanisms controlling their circadian clocks are very similar. In both, circadian rhythms control many biological processes, including cell division, hormonal release, sleep/wake cycles, body temperature and brain activity .

The researchers employed a fungus called *Neurospora,* an organism

frequently used in studies on genetics and cell processes, especially circadian rhythms. It reproduces in the dark and rests in the light.

A decade ago, Dr. Liu discovered that FRQ controlled the cellular clock in Neurospora by chemical changes of its protein structure. As the day goes on, the cell adds chemical bits called phosphates to the protein. Each new phosphate acts like a clock's ticking, letting the cell know that more time has passed.

When the number of phosphates added to FRQ reaches a certain threshold, the cell breaks it down, ready to start the cycle again.

The researchers, however, did not know where the phosphates attached to FRQ, how many got added throughout a day, or how they affected the protein's ability to "tell" time.

In the current study, the researchers used purified FRQ to analyze the specific sites where phosphate groups attach. In all, the researchers found 76 phosphate docking sites.

"This is an extremely high number," Dr. Liu said. "Most proteins are controlled by only a handful of phosphate sites."

They also studied how these phosphates are added to FRQ daily and found that two enzymes are responsible for adding most of the phosphate groups in *Neurospora*. They also found that the total number of phosphates oscillates robustly day by day.

In addition, the researchers created a series of mutations in many of the phosphate docking sites, creating strains of mold that had abnormally short or long daily clocks.

In upcoming studies, the researchers plan to identify which enzymes add

phosphates to specific sites and exactly how changes in a particular site affect a cell's clock.

Source: UT Southwestern Medical Center (news : web)