Transcription runs like clockwork

September 3 2012

(Phys.org)—It's not just a few key genes and proteins that cycle on and off in humans in a 24-hour circadian pattern as the sun rises and falls. Thousands of genes in organs throughout the body show predictable daily fluctuations, and their cycles of activity are controlled in a complex variety of ways, Howard Hughes Medical Institute researchers have discovered.

At the core of the new discovery is the finding that the function of the enzyme that transcribes genes so that they can be made into proteins—RNA polymerase—varies according to the circadian cycle. The study was published online August 30, 2012, by the journal *Science*.

Understanding how genes are cycled on and off throughout the day is key to understanding a number of biological functions, including human sleep and metabolism, says HHMI investigator Joseph S. Takahashi of the University of Texas Southwestern Medical Center. "If you look at the targets of these circadian genes, the top category is metabolic pathways. The clock is intimately involved in controlling metabolism on a daily basis."

"This finding gives us a new picture of the temporal dynamics of transcription," he says. "It gives us a new and interesting way to look at circadian cycles as well as polymerases and transcription in general."

Takahashi has been studying the circadian gene Clock and its protein product since he discovered it in the 1990s. He and others have established that CLOCK and two other proteins, BMAL1 and NPAS2,

bind to genes during the day to activate them, whereas four other circadian regulators, the proteins PER1, PER2, CRY1, and CRY2, repress genes during the night.

Takahashi and his colleagues have wanted a global view of how these activators and repressors work together to maintain the body's 24-hour rhythms. So they undertook an in-depth study of where in the genome these regulatory proteins bound to target genes in the liver cells of mice. When they conducted their search, the team was surprised to find more than 20,000 sites that one or more of the proteins bound to. At more than 1,000 of those sites, all seven proteins could bind, but many of the sites were targets for either circadian activators or repressors, not both. That was a surprise too, Takahashi says. "We naively had thought that they would all just bind to the same locations."

To determine how binding of the circadian proteins affected gene activity, the scientists went on to test the daily patterns of expression for all genes that are active in the liver.

To begin producing a protein from an active gene, cells first transcribe the information in that gene into RNA—so the amount of RNA corresponding to a particular gene can be used to measure gene activity. Before an RNA molecule is used to produce a protein, however, the RNA molecule must undergo some processing, which can influence how much protein will be produced. As part of this RNA processing, cells must excise interrupting portions of the code, known as introns. The remaining segments, known as exons, contain the essential information for building the protein specified by the gene.

To learn more about how circadian genes are regulated, Takahashi's team measured the presence of exon RNA and intron RNA in their cells separately.

If the gene expression cycles were being controlled entirely at the level of transcription, exon and intron RNA would always increase and decrease at the same time. But the researchers found something different. More than 2,000 genes showed daily cycles of expression at the exon level, but fewer than 1,400 genes showed circadian patterns at the intron level. Moreover, the intron RNA transcripts that cycled all peaked at the same time, whereas the exon RNA transcript peaks were scattered throughout different times of day.

"When we compared the intron- and exon-cycling gene sets, we found very little overlap," Takahashi says. "Only about 22 percent of the exon-cycling genes are being regulated at the level of transcription." For the other 78 percent of exon-cycling genes, the increases and decreases must be happening at a later level of regulation, rather than the initial transcription of DNA to RNA, since the intron and exon RNA transcripts don't match up.

To delve further into how regulation is occurring in the genes that do have cycling at the transcription level, and figure out why they are all peaking at the same time, Takahashi and his colleagues tested the timing of the first step in transcription, the binding of RNA polymerase II to the genes. That binding, he discovered, was occurring much earlier in the day than the gene transcription. CLOCK and BMAL1, the activators of transcription, recruit RNA polymerase II at the beginning of the cycle, but are repressed by the presence of the inhibitor, CRY1. As a consequence, RNA polymerase is poised or paused for a few hours before it can begin transcription. Thus, the circadian-rhythm-dependent steps involve both RNA polymerase recruitment and the release from the poised state.

"What we ended up discovering was that RNA polymerase II initiation is circadian on a genome-wide level," says Takahashi. "Along with the global regulation of RNA polymerase II and transcription, we also found

a global regulation of chromatin state by the circadian clock. Histone proteins that are critical for maintaining the integrity of DNA were also modified extensively on a circadian basis across the genome." This suggests that virtually every gene has the potential to be modulated along with the circadian cycle, he says. The next step, he adds, is to figure out how RNA polymerase is controlled on a daily basis and what makes the polymerase pause on some genes at certain times of the day. And, of course, the question of how other RNA molecules are being regulated after transcription still remains.

More information: Abstract: www.hhmi.org/research/investigators/takahashi.html

Provided by Howard Hughes Medical Institute