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RESEARCH NEWS

FEBRUARY 01, 2008

# Plants Can Sense Midnight

It's midnight. Do you know what your plants are doing? A new study that examined the activity of one species' 22,000 genes over a complete day and night cycle showed that midnight is a special time that plants can actually distinguish. Midnight marks the start of tasks, such as growth and protein synthesis, that plants typically neglect during the day.

The study, led by Howard Hughes Medical Institute (HHMI) investigator Joanne Chory, found that the vast majority of the plant's genes, including the newly identified `midnight' genes, cycle with changing light and temperature conditions -- far more than previously thought. Chory and Todd Michael, a former post-doctoral fellow in Chory's lab at the Salk Institute for Biological Studies, said the results of their experiments both answer important questions about how plants grow and challenge assumptions about how light affect plants' daily cycles.

Chory and her colleagues published their findings in the February, 2008, issue of *Public Library of Science Genetics*. The work was done in collaboration with Ghislain Breton and Steve Kay at the University of California, San Diego (UCSD), Todd Mockler, a former postdoctoral fellow at Salk, and other researchers at Salk, UCSD, and Oregon State University.

"Just a huge amount of the genome is responding. That was a big surprise."
**- Joanne Chory**

Plants use photosynthesis to convert light energy into chemical energy. Plants cannot photosynthesize at night, so during the day, they devote their cellular machinery to little else. When the sun goes down, they switch off the genes that encode proteins used during photosynthesis and switch on the genes that express proteins involved in growth and cellular damage repair. Plant biologists have worked for many years to understand a key question: How do plants translate cues from the environment into signals that activate the right genes at the right time of day?

Part of the answer lies with the circadian clock. According to Michael, who is now an assistant professor of plant biology & pathology at Rutgers University, the clock is a set of proteins whose activation and abundance fluctuate rhythmically over a roughly 24-hour period. The clock gates certain functions to occur only at specific times, so that, for instance, the plant won't waste energy by switching on the genes that encode photosynthesis proteins at two o'clock in the morning. But the clock doesn't work in isolation--photoreceptors synchronize the clock to the cycle of day and night, and Michael's own previous work had hinted at the importance of temperature, as well.

Those stimuli affect gene expression through a network of elements built into individual genes promoters- a short stretch of DNA recognized by the proteins that transcribe DNA to RNA. The proteins, called transcription factors, cannot start this process without binding to the promoter, and the elements ensure that they can do so only at certain times of day.

The exact time that the proteins can bind depends on the DNA sequence that makes up and surrounds each gene's module - a change in this sequence can shift activation by several hours. While each module is unique, they fall into three broad categories: morning and evening, which had been found by previous studies, and midnight, which Chory and her colleagues detected for the first time. According to Michael, the genes that respond at midnight had been overlooked because in the past researchers searched for cycling genes by creating algorithms that recognized smooth fluctuations in gene activity over the course of a day—the equivalent of sea levels rising and falling through a tidal cycle. The algorithms missed any genes that didn't fluctuate smoothly.

“We reasoned that we were missing a lot of our genes because our rhythms didn't fit exactly to very smooth curves,” said Michael. So they created their own model algorithms, based not on gradual cycles but on sudden events—like dawn and dusk. These spikes, they reasoned, better mimicked the environmental cues that actually drive gene cycling.

To obtain data to plug into their models, which they dubbed HAYSTACK, the team subjected *Arabidopsis thaliana*plants to 11 different combinations of cycling or constant hot and cold and dark and light for two days. With its small size, fast growth, and small genome -- *Arabidopsis* is the standard model organism for plant biologists. Because of its ubiquity in the lab, it was one of the first organisms to have its genome fully sequenced.

Researchers can use DNA microarray technology to measure the abundance of RNA transcripts for 22,000 of 28,000 genes in *Arabidopsis*. RNA abundance is a good measure of what activities the plant is engaged in, because the more of a given protein an organism needs, the more copies of the RNA that encodes it the cell will make.

Michael, Chory, and their colleagues used microarrays to sample gene activity in the 22,000 genes every four hours over the course of their study. Each combination entrained the plants' circadian clocks in a different way, allowing the team to determine how many genes cycled in total, and also which factors were most important.

The results were striking: 89 percent of the plants' genes cycled in response to at least one of the conditions, and 50 percent cycled in response to changes in temperature alone. Both figures were far higher than had been found previously in *Arabidopsis* studies. On the other hand, they paralleled findings from recent studies in bacteria and humans, which revealed similarly extensive gene cycling. According to Chory, the results suggest that previous studies had missed many cycling genes. “Just a huge amount of the genome is responding,” said Chory. “That was a big surprise.”

Their analysis also found the new “midnight” modules. Realizing that the short sequences were key to regulating gene expression, the researchers decided to see whether they were present in other species. So they compared promoter sequences from *Arabidopsis* to the same stretches of the poplar and rice genomes—and found they matched almost perfectly. “These time-of-day transcriptional networks are completely conserved across these distantly-related plants,” said Michael. “This suggested that [the modules are] really fundamentally important,” he said.

The work represents a fundamental shift in Chory's approach to research, and one of her first forays into the burgeoning field of bioinformatics. “What I really want to know is how plants grow,” she said. “And that's a pretty complex question.”

For 20 years, she's been trying to answer it by looking at individual genes and signaling pathways; starting with plant photoreceptors called phytochromes, she's worked back to the growth genes that these receptors affect, trying to figure out how the system works. The new study, which collected genome-wide information that the researchers then mined for patterns, stands that approach on its head. “This project takes us from a `one gene at a time' approach to looking at the entire genome,” she said.

While identifying the midnight module is a step forward, Chory and her colleagues still do not know what pathway lies between receiving light or temperature information, setting the circadian clock, and creating the right proteins to bind to the module. “It's not necessarily a direct relationship,” said Chory.

“Most likely, the clock controls a certain number of genes [directly], and then those genes control yet another level of genes,” Michael said. “We didn't get into those pathways at this point.”

“How that happens is going to be pretty interesting,” said Chory. “But I don't know the answer to it yet.”