Clone collection: First comprehensive library of master genetic switches in plants

July 17 2014

Researchers have created the first comprehensive library of genetic switches in plants, setting the stage for scientists around the globe to better understand how plants adapt to environmental changes and to design more robust plants for future food security.

The collection, which took more than 8 years and $5 million to create, contains about 2,000 clones of plant transcription factors, nature's genetic on/off switches. Manipulating these transcription factors enables scientists to improve plant traits such as cold resistance or seed quantity. The research will be published July 17 in the journal *Cell Reports*.

"[Transcription factors] are like smart missiles that go into the nucleus and bind to specific sequences of DNA," said USC Dornsife College of Letters, Arts and Sciences Dean Steve Kay, corresponding author on the study. "They will regulate genes, switching them on or off, according to how that cell needs to respond to its environment."

The library's clones of these "master switches" are stored in microtiter plates, which will be distributed to scientists worldwide, helping those in the underfunded field of plant research. Of all biomedical research, the federal government spends approximately 1 percent on plant research, according to the authors.

"Given how important food is to human health, that's rather concerning," Kay said. "Most people in the U.S. aren't worried about starvation, but they are worried about dying of cancer. To those of us who stand in the aisle of the supermarket, it's hard to believe there could be anything like a food shortage. It's like climate change—it's not often right in front of your eyes."

"We wanted to make a high fidelity, gold standard collection of transcription factors that's going to serve the plant community all over the world," said Kay, who researches circadian rhythms, or the biological clock, in plants.

Jose L. Pruneda-Paz, co-first author on the paper, explained that the collection will "ultimately, help us understand at the molecular level the mechanisms of how plants work." Pruneda-Paz helped create the library as a postdoctoral researcher, first in Kay's laboratory at The Scripps Research Institute in La Jolla, California, then at the University of California, San Diego, where he is now a faculty member.

Kay elaborated: "Along the way we are going to understand the wiring—the instruction manual—for how plants grow and develop. From that knowledge base comes all the translational opportunities."

The clones in the library were taken from *Arabidopsis*, a flowering plant related to cabbage and mustard.

"You can think of *Arabidopsis* as the mouse of the botanical world," Kay said. "In the same way we learn a great deal about human biology from flies and mice, we learn a huge amount about clock biology because *Arabidopsis* is a great plant to grow in the lab."

Pruneda-Paz and Ghislain Breton, another former postdoctoral researcher in Kay's lab and co-first author of the study, recount what first led to the idea of creating a library in 2006.

The scientists were attempting to understand how plants adapt to cycles of light and dark, and zeroed in on the production of CCA1 and LHY, transcription factors that regulate *Arabidopsis* clock genes. Traditionally, researchers decipher the function of a gene by mutating the gene and seeing whether those mutations are responsible for a certain phenotype.

But when this method didn't work, the researchers decided to "use this reverse genetics approach," Pruneda-Paz explained. "Rather than trying to do a mutation to find a gene, we could clone all the transcription factors and then figure out which transcription factors bind and regulate the expression of CCA1. For this, we started this collection."

Breton and Pruneda-Paz oversaw the initial construction of the library. In 2010 when Breton left for an assistant professorship at the University of Texas, in Houston, Pruneda-Paz completed the project. To create the library, the team received grants from the National Institutes of Health (NIH) and other grants for a total of approximately $5 million.

Pruneda-Paz and Kay finished the project at UCSD and USC, where they were aided by a robotic platform that can conduct thousands of experiments per day.

"So the goal behind this was to build something that can be the foundation of many other projects," Breton said, adding that already 70 research projects in the U.S. and Europe have resulted from the library. "It will not only be used for circadian clock research, but for many other plant biology projects in the future."

One study made possible by the library was published in *Current Biology* on July 7. In the research, Kay's laboratory learned how plants regulate their gene expression in the cold. Using the library, they conducted tests isolating an interaction between two key genes—LUX and CBF1—now known to be responsible for freezing tolerance in plants.

The research showed how plants adapt to temperature changes during the normal course of the day-night cycle, and to extreme temperature change such as frost.

"We had very little idea how cold intersected with the clock and this really reinforced the idea that transcriptional regulation is key," said first author on the *Current Biology* study Brenda Chow, a research associate in Kay's lab who will soon start a position at GenBank, a genetic sequence database in Bethesda, Maryland.

"The library has been very useful across the plant community," Chow said. "Specifically for my project, it was a unique way to identify the interaction between CBF1 and LUX. It would have been very difficult to identify this any other way."

Provided by University of Southern California