STRUCTURAL BIOLOGY

The *in vivo* RNA structurome

Genome-wide, *in vivo* RNA structure probing helps reveal how RNA structure regulates gene expression.

To really understand biology, one has to travel to the inside of a cell.

RNA structure, for example, is affected by protein interactions that are often lost in an *in vitro* context. Tertiary interactions within RNAs can also go astray when RNAs are plucked from their physiological environment. "There's really only one *in vivo* condition at any given time, but there's a multitude of *in vitro* conditions that are often lab-dependent [or] protocol-dependent, and those can alter RNA folding," explains Philip Bevilacqua of Pennsylvania State University.

Though several *in vivo* methods for probing RNA structure are available, such methods have to date only been applied to study individual transcripts. And though methods for genome-wide RNA structure probing also have been reported, such methods have not been adapted for *in vivo* profiling. With the ultimate goal being to study how RNA structure changes in plants during times of stress such as drought, Bevilacqua's collaborator Sarah Assmann says they realized that "an *in vitro* dataset wasn't going to give us the kind of answers we wanted to be getting in order to correlate RNA structure with biological function."

Assmann, a plant biologist, and Bevilacqua, an RNA chemist, teamed up to develop a method they call structure-seq, which combines dimethyl sulfate (DMS) methylation with high-throughput sequencing (Ding *et al.*, 2013). DMS is a small molecule that penetrates cells, and methylates adenine and cytosine bases found in unprotected regions (such as loops, bulges, mismatches and joining regions). The methylated bases are detected through a process of reverse transcription, ssDNA ligation, PCR to generate dsDNA and finally, deep sequencing. The researchers generated *in vivo*, nucleotide-resolution secondary structural data for more than 10,000 transcripts of *Arabidopsis thaliana* seedlings, about one-third of the *Arabidopsis* transcriptome.

Looking genome-wide *in vivo* allowed the team to make new insights that would not have otherwise been possible. For one, they identified a periodic pattern of structure and nonstructure in coding sequences with high translation efficiency. For another, they observed what appear to be structure patterns associated with alternative polyadenylation and alternative splicing.

The team also found that mRNAs expressed from housekeeping genes tended to exhibit one structure that agreed well with computational prediction. On the other hand, mRNAs from genes involved in dealing with stress were poorly predicted, which suggests greater structural plasticity that allows these mRNAs to adopt multiple structures. The work is the first step in providing a method to observe how plant RNAs refold during abiotic stress, explains Assmann. "The goal is now to reapply [the method] during the stresses and compare the datasets to find which RNAs refold and why."

Both the method and the extensive dataset should be valuable for the broader RNA field. "I'm sure there are other global trends that will be revealed from people looking at our dataset and people generating their own datasets, that we haven't even thought to look for yet," says Assmann.

The team also recently reported a new method to look at structures of individual, low-abundance transcripts *in vivo* (Kwok *et al.*, 2013). This method uses *in vivo* DMS probing or selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE) coupled with ligation-mediated PCR amplification, allowing them to obtain structural information for transcripts that are too rare to be detected by other methods. "We're pretty excited about that, too, because it is also a highly enabling methodology, which now allows the community to look at many transcripts that couldn't be looked at before," notes Bevilacqua.

Assmann and Bevilacqua highlight the important roles of the postdoc, Yiliang Ding, and two graduate students, Chun Kit Kwok and Yin Tang, on both papers. "These are really talented students who contributed both to doing the experiments, and to many of the ideas," says Bevilacqua.

Allison Doerr

RESEARCH PAPERS

Ding, Y. *et al. In vivo* genome-wide profiling of RNA secondary structure reveals novel regulatory features. *Nature* doi:10.1038/nature12756 (24 November 2013).

Kwok, C.K. *et al.* Determination of *in vivo* RNA structure in low abundance transcripts. *Nat. Commun.* doi:10.1038/ncomms3971 (16 December 2013).

© 2014 Nature America, Inc. All rights reserved.