SYSTEMS BIOLOGY

The dynamic RNA world

A combination of techniques is used to measure and model RNA dynamics in dendritic cells.

Almost nothing in biology is simple. Making a molecule such as mRNA, for instance, involves a dynamic interplay of several processes: transcription, processing and degradation, to mention only some of them. Each of these steps can, in principle, be regulated to shape a biological response.

Aviv Regev and her colleagues at the Broad Institute of the Massachusetts Institute of Technology have been working on transcription for some time. In a recent study in collaboration with Ido Amit, also at the Broad Institute, and Nir Friedman, at the Hebrew University, researchers in her laboratory used several cutting-edge methodologies to investigate the effect of other processes on cellular RNA levels (Rabani *et al.*, 2011). "Our goal initially was really to look at other stuff that happens to RNA," Regev says, "and we were rooting for the idea that there might be a lot of regulation that doesn't have to do with transcription."

Previous work has suggested that mRNA degradation rates may be important for regulating transcript levels. But many studies either do not look at these effects during dynamic processes or use methods, such as treatment with actinomycin D, that compromise the health of the cells. By combining several existing technologies, Regev and colleagues overcame several of these limitations.

They pulse-labeled cells with 4-thiouridine, which allows biotinylation and capture of newly synthesized transcripts, and used highly sensitive Nanostring technology to detect the RNA. This made it possible to use very short 4-thiouridine labeling times (10 minutes) and therefore to measure transcription rates directly. Regev and colleagues applied this approach to monitor, with high temporal resolution, the dynamic profiles of newly transcribed and total RNA for 254 genes in dendritic cells responding to lipopolysaccharide.

Using a modeling approach, the researchers then inferred degradation rates by testing, for each gene, whether the experimental observations were best fit by a model with a constant versus a varying degradation rate. They found that, for the majority of genes, the simpler model with a constant degradation rate was better at describing most of the data. But for a fraction of genes, the data were better fit by a model with varying degradation rates. "It's a small but interesting fraction," says Regev, "a select club of supercool genes, and a lot of them are ones that immunologists who work on dendritic cells care about the most."

To apply their measurements genome-wide, Regev and colleagues also used Illumina sequencing to read out the 4-thiouridine–labeled RNA. In this case, they labeled the cells for a bit longer (45 minutes) and looked at profiles of almost 10,000 genes over 6 hours after addition of lipopolysaccharide, albeit at reduced temporal resolution. In this case too, the profiles of most genes could be explained by assuming a constant degradation rate.

It remains to be seen whether the principles that emerge from this study also apply to other systems, and to what extent rates of RNA processes control protein levels as well. Indeed, a recent study on unperturbed mouse fibroblasts suggested that both mRNA levels and translation rate have important roles in regulating protein levels at steady state (Schwanhäusser *et al.*, 2011).

As a fortunate consequence of using 4-thiouridine labeling for massively parallel sequencing (4sU-seq), Regev and colleagues could infer mRNA processing rates, too, and observed that they vary substantially between transcripts. Whereas most RNA-seq protocols require making poly(A)-selected libraries to remove ribosomal RNA, Regev and colleagues did not need to do so because little ribosomal RNA is transcribed in the 45 minutes used for 4-thiouridine labeling. They thus obtained a much broader view of cellular RNA species than in a typical RNA-seq experiment and, by modeling, identified a small set of transcripts that may be regulated by changes in RNA processing rates. Regev predicts that this is an area that will prove fruitful in the future.

Natalie de Souza

RESEARCH PAPERS

Rabani M. *et al.* Metabolic labeling of RNA uncovers principles of RNA production and degradation dynamics in mammalian cells. *Nat. Biotechnol.* **29**, 436–442 (2011).

Schwanhäusser B. *et al.* Global quantification of mammalian gene expression control. *Nature* **473**, 337–342 (2011).

© 2011 Nature America, Inc. All rights reserved.