GENE EXPRESSION

The human transient transcriptome

Fragmenting transcripts after 4-thiouridine incorporation allows the quantification of even short-lived noncoding RNA.

Short RNAs expressed at regulatory elements are important for the regulation of genes. Take enhancer RNAs (eRNAs), for example: their bidirectional transcription at enhancer sites strongly correlates with the active state of the enhancers, but their short half-life of only a few minutes makes profiling challenging. Without knowledge about the diversity and kinetics of these noncoding RNAs, it is difficult to elucidate their role.

Current techniques that take stock of a cell's transcript repertoire are not ideally suited to pick up transient signals in large genomes. Transcript labeling with 4-thiouridine followed by sequencing (4sU-seq) allows the enrichment of transient RNAs and the determination of their synthesis and decay, but because the labeling pulse is short, the unlabeled 5′ region of a gene is predominant in the sequencing data compared to the short labeled 3′ end. This is less of an issue in smaller genomes such as yeast, but when looking at the much larger human transcriptome, the method loses too much sensitivity.

Patrick Cramer from the Max Planck Institute in Goettingen, Germany, and Julien Gagneur, now at the Technical University in Munich, teamed up and introduced a fragmentation step after 4sU labeling to ensure enrichment of the labeled 3′ region. The challenge of transient transcriptome sequencing (TT-seq), as co–first author Margaux Michel, a PhD student in the Cramer lab, explains, was fine-tuning the fragmentation to be as sensitive as possible while not losing too much RNA.

TT-seq data on human cells recovered many transcriptional units that had not been annotated before. The team grouped them according to their position relative to known transcription start sites and surrounding chromatin signals. They mapped 2,500 short intergenic RNAs to promoters and found just over 3,000 eRNAs. Kinetic modeling showed that mRNAs and long intergenic noncoding RNAs have the highest synthesis rates and the longest half-lives, with a median of 50 minutes, whereas eRNA half-lives span just a few minutes. Transient RNAs that mapped downstream of poly A sites allowed the researchers to find new transcription termination sites.

Having a way to capture these short-lived regulatory RNA species will provide a window into their activation and degradation as well as their roles during transcriptional events such as splicing.

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RESEARCH PAPERS

Schwalb, B., Michel, M. *et al.* TT-seq maps the human transient transcriptome. *Science* **352**, 1225–1228 (2016).