SEQUENCING

# Rereading familiar messages

**Two surveys of the transcriptome hint at unexpected diversity in the breadth of mRNA modifications.**

Every RNA is initially composed of a relatively small roster of familiar characters—adenine, guanine, cytosine and uracil—but specialized enzymes can subsequently expand the cast to include more exotic modified nucleosides such as pseudouridine (ψ). However, almost all of these have been found exclusively in rRNA and tRNA, with only a handful observed in mRNA.

"We know there are over 100 modifications that can occur," says Schraga Schwartz, a postdoc in the lab of Aviv Regev at the Massachusetts Institute of Technology (MIT). "They could also be on mRNA, and we just don't know because we haven't looked carefully enough." Two research teams at MIT—one led by Regev and Gerald Fink (Schwartz *et al.*, 2014) and the other by Wendy Gilbert (Carlile *et al.*, 2014)—have now independently discovered that numerous mRNAs do in fact undergo ψ modification and that the patterns of those modifications can vary depending on a cell's physiological state.

Both groups built their discovery strategy around a technique developed over 20 years ago, subjecting purified RNA to a chemical treatment that selectively modifies ψ nucleosides. This alteration acts as a block to the reverse-transcriptase enzyme, and high-throughput sequencing of the resulting reactions can thus reveal the precise location of ψ sites throughout the transcriptome. Working with yeast, the two teams detected virtually all of the known modifications in rRNA and tRNA, as well as striking evidence that a small subset of mRNA transcripts also contain ψ at specific sites.

Yeast perform this modification with nine different pseudouridine synthase (PUS) enzymes, and the two research teams each combined informatic analysis with a series of deletion mutants to match the various mRNAs with the enzyme that modifies them. "Those enzymes are essentially universally conserved," says Gilbert. "Since we found that all of them have the capacity to modify mRNAs, my guess is that some mRNAs are modified under certain growth conditions in every organism." Indeed, both groups could detect numerous ψ-modified mRNAs in the human transcriptome as well.

Subjecting human or yeast cells to different growth conditions strikingly altered the RNA modification profile. For example, Schwartz and co-lead author Douglas Bernstein (a postdoc in the Fink lab) found 265 RNA sites in yeast that specifically undergo ψ modification in response to heat shock and determined that these generally appear to undergo modification via the same PUS enzyme. Gilbert's team likewise identified specific changes associated with different cellular growth states or nutrient availability. "Overall, the stories are very complementary," says Gilbert. "And because they looked at a different stress from us, this suggests that it could be a very rich regulatory landscape."

These findings might also offer insights into certain human diseases. "We looked at cells from dyskeratosis congenita patients, who have a mutation in a specific PUS, and saw a 10% decrease in pseudouridinylation levels in these patients," says Bernstein. Owing to limitations in the method's sensitivity, these changes were detectable in only non-mRNA targets, but Schwartz hopes to perform a deeper survey in future research. Similarly, Gilbert notes that an RNA that helps one of the PUS enzymes to recognize its targets has been linked to tumor progression, and she plans to examine whether abnormal ψ modification of mRNAs may play a role. This will also entail clarifying the specific function of ψ; most evidence to date suggests that it confers additional stability to rRNA and tRNA, although some studies have raised the intriguing possibility that ψ could potentially affect the translation of modified mRNAs.

Gilbert is especially excited by the chance to uncover other 'missing' modifications. "If some mRNAs look like tRNAs to one class of tRNA-modifying enzymes, who's to say they don't get modified by others?" she says. "The landscape of mRNA modifications could be an order of magnitude richer than we currently realize."

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**RESEARCH PAPERS**

Carlile, T.M. *et al.* Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. *Nature* doi:10.1038/nature13802 (5 September 2014).

Schwartz, S. *et al.* Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. *Cell* **159**, 148–162 (2014).