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RNA

Pseudouridylation of mRNAs — not so pseudo


CORBIS

Two new reports, published in *Nature* and *Cell* within the same week, describe the independent development of high-throughput techniques, named Pseudo-seq and Ψ-seq, respectively, for the transcriptome-wide identification of pseudouridylation sites. Both studies reveal a high abundance of pseudouridines (Ψ) in mRNAs of yeast and humans.

Pseudouridylation, which is the most prevalent post-transcriptional RNA modification, has been shown to increase the stability of tRNAs and ribosomal RNAs. Additionally, loss of pseudouridine synthases — the enzymes that mediate pseudouridylation — has profound effects in a wide range of organisms, including bacteria, yeast and humans. The artificial pseudouridylation of mRNAs suggested that this modification acts to increase the diversity of the genetic code and provided proof-of-principle that this post-transcriptional modification can drastically affect the function of mRNAs, although natural mRNAs that contain pseudouridines remained elusive.

The investigators opted for high-throughput approaches based on primer-extension assays pioneered by Bakin and Ofengand. "This method relies on the fact that pseudouridine can be selectively derivatized with a bulky carbodiimide that causes reverse transcriptase to stop one nucleotide 3′ to the pseudouridine," explains Wendy Gilbert, who led the study published in *Nature*. "We adapted their approach to transcriptome-wide pseudouridine profiling by replacing gene-specific primers with a universal priming strategy and replacing sequencing gels with next-generation sequencing." In addition to known pseudouridines in non-coding RNAs, the two studies identified hundreds of unknown sites of pseudouridylation in both non-coding and coding RNAs of yeast cells and human cells. These sites were found to be under dynamic regulation, for example, in response to stress such as nutrient deprivation or heat shock. The researchers also linked individual pseudouridylation sites to one of several pseudouridine synthases by using Pseudo-seq or Ψ-seq to profile the transcriptome of yeast strains lacking specific pseudouridine synthases.

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The teams of Gerald Fink and Aviv Regev, who collaborated on the *Cell* study, further investigated pseudouridylation in two patients with X-linked dyskeratosis congenita, which is a disorder caused by mutations in the pseudouridine synthase gene *DKC1*. rRNA pseudouridylation was subtly but significantly decreased in patients relative to age-matched controls, as was pseudouridylation of the non-coding telomerase RNA component (TERC). Mutations in TERC can also result in dyskeratosis congenita. "The association of this human disease, which is characterized by a predisposition to cancer and bone marrow failure, with mutations in a pseudouridine synthase gene suggests that Ψ-seq has applications in uncovering the significance of RNA pseudouridylation in human pathologies," comments Fink.

Taken together, the findings raise a host of questions regarding the function of pseudouridines in mRNAs. "Perhaps the most exciting prospect is regulated 'rewiring' of the genetic code," suggests Gilbert. "We are actively pursuing this using targeted mass spectrometry to look for re-coded peptides encoded by pseudouridylated mRNAs." Other promising avenues of investigation are the impact of pseudouridylation on gene regulation, whether through effects on translation, mRNA stability or RNA localization, as well as the mechanisms underlying the dynamic regulation of mRNA pseudouridines in response to stress and developmental cues.

Linda Koch

ORIGINAL RESEARCH PAPERS Carlile, T. M. *et al.* Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. *Nature* http://dx.doi.org/10.1038/nature13802 (2014) | Schwartz, S. *et al.* Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. *Cell* http://dx.doi.org/10.1016/j.cell.2014.08.028 (2014)