Mim-tRNAseq: A method that accurately measures the abundance and modification status of different tRNAs

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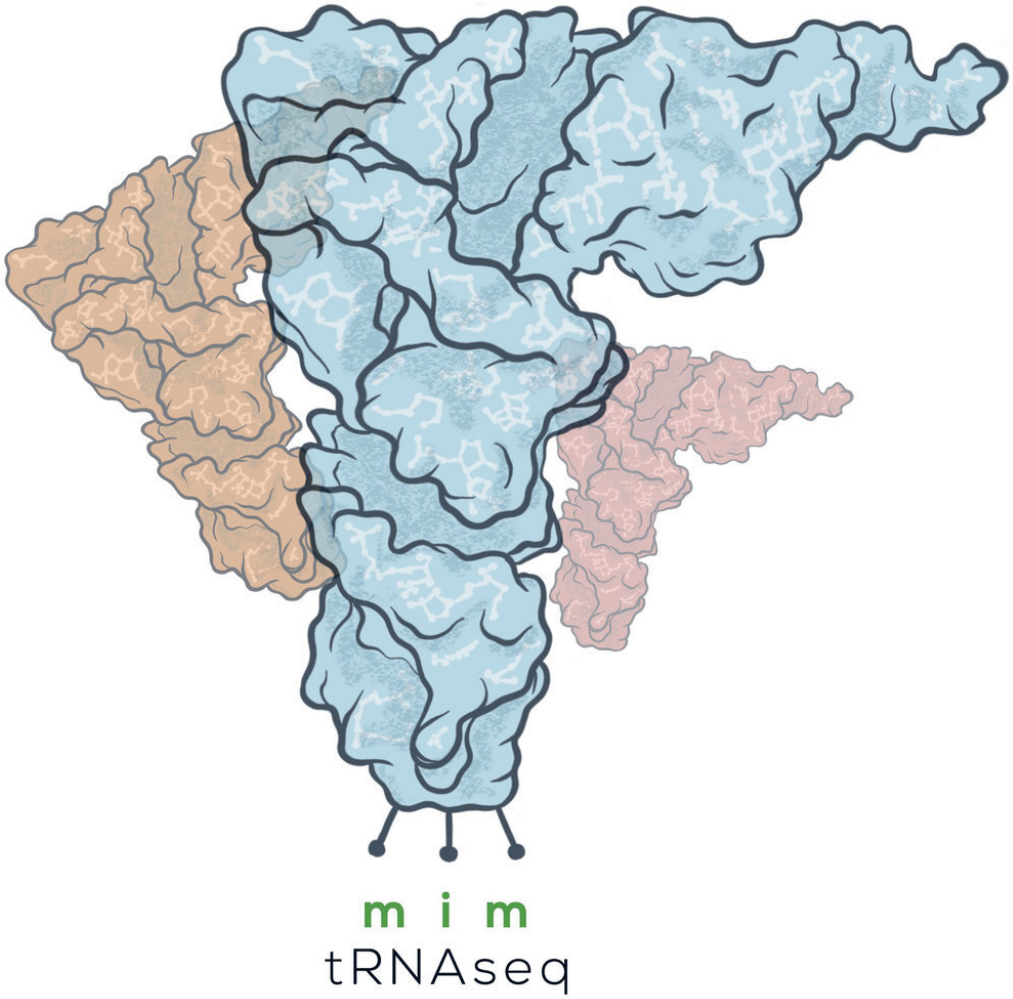

Artistic representation of tRNAs © Heather Jenkins

Transfer RNAs (tRNAs) deliver specific amino acids to ribosomes during translation of messenger RNA into proteins. The abundance of tRNAs can therefore have a profound impact on cell physiology, but measuring the amount of each tRNA in cells has been limited by technical challenges. Researchers at the Max Planck Institute of Biochemistry have now overcome these limitations with mim-tRNAseq, a method that can be used to quantify tRNAs in any organism and will help improve our understanding of tRNA regulation in health and disease.

A cell contains several hundred thousand tRNA molecules, each of which consists of only 70 to 90 nucleotides folded into a cloverleaf-like pattern. At one end, tRNAs carry one of the twenty amino acids that serve as protein building blocks, while the opposite end pairs with the codon specifying this amino acid in messenger RNA during translation. Although there are only 61 codons for the 20 amino acids, cells from different organisms can contain hundreds of unique tRNA molecules, some of which differ from each other by only a single nucleotide. Many nucleotides in tRNAs are also decorated with chemical modifications, which help tRNAs fold or bind the correct codon.

The levels of individual tRNAs are dynamically regulated in different tissues and during development, and tRNA defects are linked to neurogical diseases and cancer. The molecular origins of these links remain unclear, because quantifying the abundance and modifications of tRNAs in cells has long remained a challenge. The team of Danny Nedialkova at the MPI of Biochemistry has now developed mim-

tRNAseq, a method that accurately measures the abundance and modification status of different tRNAs in cells.

Modification roadblocks and resolutions

To measure the levels of multiple RNAs simultaneously, scientists use an enzyme called reverse transcriptase to first rewrite RNA into DNA. Millions of these DNA copies can then be quantified in parallel by high-throughput sequencing. Rewriting tRNAs into DNA has been tremendously hard since many tRNA modifications block the reverse transcriptase, causing it to stop synthesizing DNA.

"Many researches have proposed elegant solutions to this problem, but all of them relieve only a fraction of the modification roadblocks in tRNAs," explains Danny Nedialkova, Max Planck Research Group Leader at the Max Planck Institute of Biochemistry. "We noticed that one specific reverse transcriptase seemed to be much better at reading through modified tRNA sites. By optimizing the reaction conditions, we could significantly improve the enzyme's efficiency, enabling it to read through nearly all tRNA modification roadblocks," adds Nedialkova. This made it possible to construct DNA libraries from full-length tRNA copies and use them for high-throughput sequencing.

The mim-tRNAseq computational toolkit

The analysis of the resulting sequencing data also presented significant challenges. "We identified two major issues: the first one is the extensive sequence similarity between different tRNA transcripts," explains Andrew Behrens, Ph.D. student in Nedialkova's group and first author of the paper. "The second one comes from the fact that an incorrect nucleotide (a misincorporation) is introduced at many modified sites during reverse transcription. Both make it extremely challenging to

assign each DNA read to the tRNA molecule it originated from," adds Behrens.

The team tackled these issues with novel computational approaches, including the use of modification annotation to guide accurate read alignment. The resulting comprehensive toolkit is packaged into a freely available pipeline for alignment, analysis and visualization of tRNA-derived sequencing data . Researchers can use mim-tRNAseq to not only measure tRNA abundance, but also to map and quantify tRNA modifications that induce nucleotide misincorporations by the reverse transcriptase. "mim-tRNAseq opens up myriad possibilities moving forward," says Nedialkova. "We expect it will help us and others to tackle many outstanding questions about tRNA biology in health and disease."

More information: Andrew Behrens et al, High-resolution quantitative profiling of tRNA abundance and modification status in eukaryotes by mim-tRNAseq, *Molecular Cell* (2021). DOI: 10.1016/j.molcel.2021.01.028

github.com/nedialkova-lab/mim-tRNAseq

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