DECODING THE EPITRANSCRIPTOME: HARNESSING SEQUENCING TECHNIQUES FOR UNDERSTANDING RIBOSOME HETEROGENEITY

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The epitranscriptome refers to the chemical modifications of RNA which are known to regulate RNA stability, translation, and processing, playing key roles in gene expression and cellular function. Ribosomes, major players in protein synthesis, are subject to extensive rRNA modifications which contribute to the so-called ribosome heterogeneity, i.e. the existence of distinct populations of ribosomes with diverse compositions, conformations, and activities. Among the rRNA modifications, the 2'O-methylation (2'Ome) is the most widespread. 2'Ome influence ribosome biogenesis and translation fidelity, potentially adding a translation regulatory layer under physiological and pathological conditions. Traditionally, approaches such as Cryo-EM, NMR and mass-spectrometry have been widely applied for obtaining atomic-resolution insights in 2′-O-methylated nucleotides within the ribosome structure. However, these approaches face technical challenges and do not allow neither high-throughput analyses nor the quantification of rRNA methylation levels. Complementary approaches are required for the comprehensive understanding of heterogeneous ribosomes.

We exploited RiboMethSeq, a next-generation sequencing-based technique, to resolve at single nucleotide resolution the rRNA 2'Ome landscape in the context of the Spinal Muscular Atrophy. SMA is a genetic disease which impact newborn development due to low levels of the Survival Motor Neuron (SMN) protein. SMN is known to be implicated in translation regulation, though the mechanisms remain unclear. Prompted by the established interaction of SMN with Fibrillarin, the protein responsible for the catalysis of 2'Ome, we investigated the role of SMN as modulator of rRNA modifications. Through RiboMethSeq, we quantified the 2'Ome levels in SMA early-symptomatic mouse brain compared to healthy individuals. Interestingly, we observed multiple changes at rRNA sites reported to show decreasing levels of methylation during development, reinforcing their connection with SMA. Exploiting published Cryo-EM ribosome structures, we located one altered site close to regions involved in translations elongation and initiation, supporting SMN's potential role as modulator of rRNA modifications and translation.

Overall, our findings emphasize how sequencing techniques and specialized computational pipelines effectively extract valuable insights into epitranscriptome and ribosome heterogeneity at single-nucleotide resolution.