BIOINFORMATICS

RNase footprints re-examined

A computational tool enables the study of native nonribosomal RNA–protein complexes from ribosome profiling data.

Ribosome profiling experiments, in which ribosome-associated RNA is enriched and sequenced, have illuminated our understanding of translation in a range of biological settings. Now, through the use of a new computational tool, these data sets can be repurposed for the study of nonribosomal RNA–protein complexes transcriptome-wide.

Knowledge of nonribosomal RNA–protein complexes can shed light on the function of RNAs and their regulation in the cell. Several existing methods for such studies rely on cross-linking of RNA and protein, but complexes that are identified in this way do not necessarily represent native complexes. In addition, these methods use peak calling to define protein-bound RNA sequences, and thus they cannot be used to determine the presence of multiple RNA–protein complexes in the same RNA region.

The new Rfoot pipeline, developed by Kevin Struhl at Harvard University, Aviv Regev at the Broad Institute of MIT and Harvard, and their colleagues, mines data from ribosome profiling experiments, in which large complexes (>100–200 kDa) are enriched by sucrose gradient after RNase treatment. The remaining RNA is sequenced and represents the ribosome-bound fraction that is protected from digestion. However, Rfoot analysis reveals that 11.3% of these sequence reads correspond to RNA regions protected by nonribosomal complexes.

Rfoot searches for regions that do not show three-nucleotide periodicity, a trademark of ribosome binding, and that are highly localized, as would be expected of nonribosomal RNA–protein binding. When the researchers applied the algorithm to ribosome profiling data from two isogenic human cell lines, they found previously unknown RNase-protected regions in all RNA species studied, including long noncoding (lnc) RNAs, small nucleolar (sno) RNAs, microRNAs, tRNAs and the 3′ UTR of mRNAs.

The picture that emerges from this initial analysis suggests that the conformation and/or the stability of nonribosomal RNA–protein complexes varies even for RNAs of the same class. Interesting findings regarding lncRNAs, such as RNase footprints that differ in the two cell lines studied and lncRNAs with multiple and distinct protected regions, would merit follow-up studies. These results promise many more discoveries to come as ribosome profiling data sets are reanalyzed using Rfoot.

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RESEARCH PAPERS

Ji, Z., Song, R., Huang, H., Regev, A. & Struhl, K. Transcriptome-scale RNase-footprinting of RNA-protein complexes. *Nat. Biotechnol.* **34**, 410–413 (2016).