Trends in Biochemical Sciences | Technology of the Month

Approaches for Studying Ribosome Specialization

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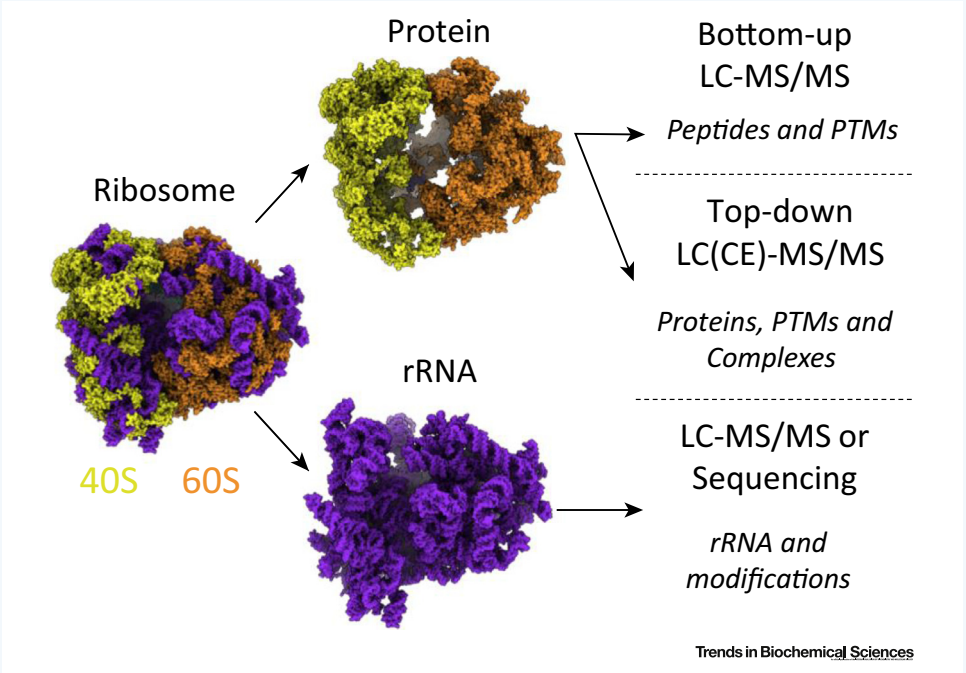
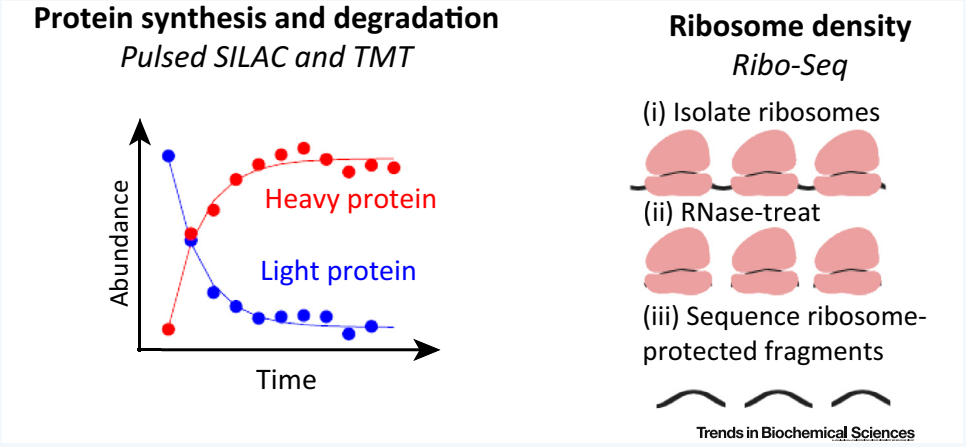


Contrary to the textbook model, recent measurements demonstrated unexpected diversity in ribosomal composition that likely enables specialized translational functions. Methods based on liquid chromatography coupled to tandem mass-spectrometry (LC-MS/MS) enable direct quantification of ribosomal proteins with high specificity, accuracy, and throughput. LC-MS/MS can be 'top-down', analyzing intact proteins, or more commonly 'bottom-up', where proteins are digested to peptides prior to analysis. Changes to rRNA can be examined using either LC-MS/MS or sequencing-based approaches.

The regulation of protein synthesis by specialized ribosomes can be examined by multiple methods. These include the popular 'Ribo-Seq' method for analyzing ribosome density on a given mRNA, as well as LC-MS/MS approaches incorporating pulse-labelling with stable isotopes (SILAC) to monitor protein synthesis and degradation.



ADVANTAGES:

Bottom-up LC-MS/MS: high specificity, accuracy, and throughput. Robust protocols and commercially available reagents. Pulsed SILAC/tandem mass tags (TMT): nonradioactive and high proteome coverage with reduced missing data compared with pulsed-SILAC alone.

Top-down LC-MS/MS: complete coverage of proteoforms. Ability to quantify complexes.

Sequencing: widely available instrumentation and methods. Long-read and direct RNA sequencing approaches. Ribo-Seq: can provide nucleotide-level detail on translation, including frameshifting and ribosome pausing events.

CHALLENGES:

Bottom-up LC-MS/MS: incomplete protein coverage → use multiple proteases. Biases in quantification → use both methods that derive quantification from the survey mass-spec scans (MS1) [e.g., SILAC, mass differential tags for relative and absolute quantification (mTRAQ), dimethyl-labeling] and methods that derive quantification from ion fragments measured in the tandem mass-spec scans (MS2/MS3) [e.g., TMT, isobaric tags for relative and absolute quantitation (iTRAQ)]. Difficulties in proteoform analysis → use computational approaches. Pulsed SILAC/TMT: difficulties in analyzing combined MS1/MS2 quantification results → improve analysis software.

Top-down LC-MS/MS: limited mass spectrometer resolution → use advanced instrumentation. Low sensitivity → improve delivery and concentrate samples. Complex sample handling and analysis → need for robust methods and improved access to instrumentation and software.

Sequencing: difficulties in analyzing rRNA due to poor rDNA coverage in genome assemblies → new analysis methods. Ribo-Seq: large RNA input amounts and sequence-dependent biases → increase input or sequence intact mRNAs from sucrose fractions.

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  https://doi.org/10.1016/j.tibs.2019.01.008

Acknowledgments

This work was funded by a New Innovator Award from the NIGMS from the National Institutes of Health to N.S. under Award Number DP2GM123497 and a MIRA award from NIGMS from the National Institutes of Health to M.J. under Award Number R35GM128802.

Literature

1. Slavov, N. *et al.* (2015) Differential stoichiometry among core ribosomal proteins. *Cell Rep.* 13, 865–873
2. Emmott, E. *et al.* (2019) Ribosome stoichiometry: from form to function. *Trends Biochem. Sci.* 44, 95–109
3. Popova, A.M. and Williamson, J.R. (2014) Quantitative analysis of rRNA modifications using stable isotope labeling and mass spectrometry. *J. Am. Chem. Soc.* 136, 2058–2069
4. Parks, M.M. *et al.* (2018) Variant ribosomal RNA alleles are conserved and exhibit tissue-specific expression. *Sci. Adv.* 4, eaao0665
5. Ingolia, N.T. (2014) Ribosome profiling: new views of translation, from single codons to genome scale. *Nat. Rev. Genet.* 15, 205–213
6. Zecha, J. *et al.* (2018) Peptide level turnover measurements enable the study of proteoform dynamics. *Mol. Cell. Proteomics* 17, 974–992
7. Garalde, D.R. *et al.* (2018) Highly parallel direct RNA sequencing on an array of nanopores. *Nat. Methods* 15, 201–206
8. Sinitcyn, P. *et al.* (2018) Computational methods for understanding mass spectrometry–based shotgun proteomics data. *Annu. Rev. Biomed. Data Sci.* 1, 207–234
9. Malioutov, D. *et al.* (2018) Quantifying homologous proteins and proteoforms. *Mol. Cell. Proteomics* 18, 162–168
10. Belov, A.M. *et al.* (2017) Analysis of proteins, protein complexes, and organellar proteomes using sheathless capillary zone electrophoresis – native mass spectrometry. *J. Am. Soc. Mass Spectrom.* 28, 2614–2634

  https://doi.org/10.1016/j.tibs.2019.01.008