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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[50_TD\$DIFF], as well as LC-MS/MS approaches incorporating pulse-labelling with stable isotopes (SILAC) to monitor protein synthesis and degradation.

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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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