PROTEO-TRANSLATOMICS:
WHEN PROTEOMIC AND TRANSLATOMICS MERGE TOGETHER

Protein expression at a given time determines the cellular states. mRNA and protein expression are tightly linked and finely regulated by transcripts and proteins production/degradation. A multitude of studies using quantitative proteomics in conjugation with transcriptomic has shown that globally there is a poor correlation between mRNA and protein levels in various biological systems (1, 2, 3). It has become evident that translational control appears to be critical in determining the steady-state levels of most cellular proteins.

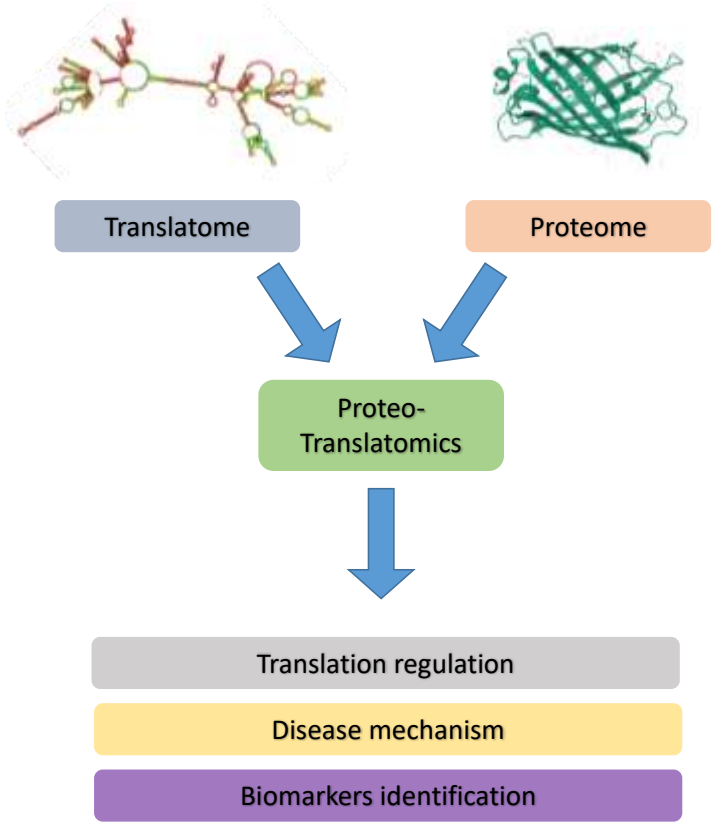

Figure 1. The concept of Proteo-Translatomics. Proteomic and Translatomics data are used to investigate gene expression.

As a result, interest in identify mRNAs associated with ribosomes (translatome) to study translational regulation processes has increased significantly in recent years. Global translatome analysis techniques, such as Polysome Profiling (quantification of mRNA abundance in polysome fractions purified by sucrose gradient), (4), ribosome profiling (isolation and sequencing of ribosome-protected mRNA fragments), (5) and Translating Ribosome Affinity Purification TRAP (isolation of tagged polysomes and associated RNAs) (6), has been widely applied to study how translational regulation of gene expression controls cellular processes.

This has been accompanied by the development of a plethora of different methodologies that enable the evaluation of protein translation in vitro and in vivo. The advent of Mass spectrometry has open the door to the study of the protein level as well as the investigation of the protein post-translation modifications. The gold standard in proteomic is the pulse labelling with “heavy” amino acid (pSILAC). This technique is based on the

incorporation of amino acids with “heavy” stable isotopes and allows to discriminate between newly synthesized and pre-existed proteins (7). Recently, another tool for the identification of newly synthesized proteins was developed: Biorthogonal Noncanonical amino acid tagging (BONCAT) (8). This technique is based on the incorporation of a “clickable” group obtained through replacement of the methionine amino acid by methionine-analogues homopropargylglycine (HPG) or azidohomoalanine (AHA). The reactivity of these lateral groups enables the covalent functionalization of the *de-novo* synthesized proteins with specific probes that, following protein digestion and beads purification, allows MS identification of alkyne/azide containing peptides. The main advantage of this technique respect to the SILAC is the possibility to strongly decrease the incubation time required for the labelling, strongly reducing the windows of analysis of the newly synthesized proteins.

The process of identifying all coding regions in a genome is crucial for any study at the level of molecular biology, ranging from single-gene cloning to genome-wide measurements using RNA-seq or mass spectrometry. While satisfactory annotation has been made feasible for well-studied model organisms through great efforts of big consortia, for most systems this kind of data is either absent or not adequately precise. An integrated translatome analysis and proteome analysis can help in a more accurate depiction of translational control mechanism, essential in understanding cell homeostasis and cellular diseases.

Immagina BioTechnology s.r.l. provides uniquely enabling technologies to break down walls on proteo-translatomics and ribosome profiling studies.

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