Mass spectrometry and ribosome profiling, a perfect combination towards a more comprehensive identification strategy of true in vivo protein forms.

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INTRODUCTION

An increasing number of studies involve integrative analysis of gene and protein expression data, taking advantage of new technologies such as next-generation transcriptome sequencing (RNA-Seq) and high sensitive mass spectrometry (MS). Recently, a strategy, termed ribosome profiling, based on deep sequencing of ribosome-protected mRNA fragments, indirectly monitoring protein synthesis, has been described. When used in combination with identification-specific translation inhibitors, it enables the identification of (alternative) translation initiation sites.

In contrast to routinely employed protein databases in proteomics searches, Ribo-seq derived data gives a more representative expression state and accounts for sequence variation information (single nucleotide polymorphisms, insertions, deletions and RNA-splice variants) and alternative translation initiation leading to N-terminal extended and/or truncated protein forms. Furthermore, Ribo-seq reveals translation start at near-cognate start sites. Without taking this information into account, MS-based proteomic studies may fail to detect novel, important protein forms.

GOALS

 ✓ Compile a sample-specific protein search database based on ribosome profiling sequencing data.
 ✓ Introduce new translation products in the MS search space: N-terminal extensions/truncations, trans-labeled uORFs, near-cognate start sites.
 ✓ Bridging two omics worlds: transcriptomics & MS-based proteomics by means of Ribo-seq.

RESULTS

CONCLUSION & FUTURE WORK

 ✓ Deep proteome coverage based on ribosome profiling aids mass spectrometry-based protein and peptide discovery and provides evidence of alternative translation products and near-cognate translation initiation events [7].
 ✓ Future work will mainly focus on:
   ✓ Further investigation of the differential expression on translation level of UniProt/SwissProt and Ribo-seq derived translation products technological and/or biological relevance. Detailed assessment of the difference between in vivo measurement of protein (Ribo-seq) and protein presence (MS-based proteomics).
   ✓ Generalize the pipeline to all types of next-generation sequencing RNA-seq data: [directional] A+/A- RNA-seq, CLIP-seq, exome-seq, ribo-seq.
   ✓ Quantitative correlation of Ribo-seq and (non) labelled MS-based proteomics
   ✓ Incorporate Pipeline into Galaxy-P

REFERENCES


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