Untangling protein synthesis with ribosome profiling

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We used ribosome profiling (ribo-seq) to assess the gene expression response of mammalian cells to various stresses, such as increased eIF2 phosphorylation (the key step in Integrated Stress Response)[1], and Oxygen and Glucose Deprivation (OGD)[2]. It enabled us to delineate the rapid translational response affecting thousands of genes. The response frequently involves translation of short regulatory ORFs usually located in the 5' leaders of mRNAs. We also observed translation of unannotated long ORFs that likely leads to the synthesis of novel protein products specific to stress conditions.

To assist the research community in using ribo-seq data we are developing RiboSeq.Org suite of tools (http://riboseq.org) that currently consists of the GWIPS-viz browser for the visualization of genomic alignments of ribosome footprints[3] and RiboGalaxy which is a Galaxy instance specifically tailored for the analysis of ribo-seq data[4].

In addition we developed a simple computational approach for the characterization of ribo-seq datasets[5]. This technique is resistant to irregular technical noise and aberrant footprint densities caused by ribosome pauses. Application of this approach to several ribo-seq datasets revealed the strong impact of sequencing biases and translation inhibitors on the distribution of aligned ribosome footprints as well as substantial non-biological variability between datasets obtained from different laboratories.

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