Elucidating the impact of upstream open reading frames on translational regulation

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High-throughput sequencing has revealed evidence for pervasive transcription of the genome. The majority of these regions are considered to be untranslated and non-coding. Ribosomal profiling assay allows precise identification of the actively translated transcripts. Specifically, ribosomal footprints can be detected as ribosomes protect bound RNA fragments from nuclease digestion. Our group developed a computational method (RiboTaper) that identifies open reading frames (ORF) exhibiting ribosomal occupancy consistent with translation in the annotated coding and non-coding regions of the genome. In particular, RiboTaper can identify upstream ORFs (uORFs) – short ORFs in the 5'UTRs of the coding genes. uORFs are typically associated with translational repression of the main ORFs, particularly in response to stress conditions. However, the function of most uORFs remains unclear.

In this study we focus on uORFs located upstream translation initiation factors (EIFs). We applied our method to the ribosomal profiling data from drosophila, zebrafish, mouse, rat and human cells and identified uORFs with similar position and length present in several EIFs homologous in the majority of species. We hypothesize that EIFs regulate their own translation by interacting with uORFs or their corresponding peptides, thus creating an autoregulatory translational loop. To test our hypothesis, we designed a reporter system with functionally active and inactive uORFs and will evaluate the translational output of EIFs under normal and stress conditions. In addition, we will analyze available ribosomal profiling data from cells exposed to various stress at different time points. Along with that analyzing ribosomal sequencing data from ~70 individuals may provide novel insights in the variability of the uORF sequences and positions relative to the main ORFs and their impact on EIFs translation. We expect to reveal novel mechanisms of translational regulation in eukaryotes.