Translational Regulation Mechanisms of uORFs

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Last semester the control of mRNA translation during transition from one stage to another – such as one adapted for stress conditions – was vital to understanding the regulation of gene regulation in human cells including cancer cells, yet is not fully understood. Cellular signaling controls translation by modulating mRNA recruitment through cis regulatory elements or utilizing delayed re-initiation mechanisms involving upstream ORFs (uORF). Typically, the regulated mRNAs encode transcription factors, allows genome-wide transcriptional responses. Amino acid starvation activates Gcn2 eIF2a kinases. eIF2a phosphorylation caused slow growth partly through inhibition of protein synthesis, and yet facilitated translation of hundreds of specific mRNA species. Expression of resistance to a metabolic stress like this one is crucial for cancer cells to establish themselves during their migration and metastasis.

Using fission yeast Schizosaccharomyces pombe as a model organism, we used the polysomal microarray hybridization experiments to reveal hundreds of candidate genes whose translation is stimulated by amino acid starvation in Gcn2-dependent manner. Their functions included mitochondrial translation, mRNA splicing, tRNA production, and mRNA transcription including histone acetyltransferases and methyltransferases. Of particular interest was that the gene list contained many histone acetyltransferase genes including Gcn5, which is known to promote starvation response in this yeast. These and other listed genes, such as Hri2 encoding heme-regulated eIF2a kinase, possess multiple uORFs reminiscent of regulation by delayed re-initiation. Moreover, bioinformatics analysis indicated that certain RNA motifs were enriched in translationally controlled genes. They included overlapping motifs that commonly contain a UGA(C/A)G sequence, reminiscent of the GAC motif characterized as N_6 adenosine methylation (m6A) site for the adenine residue in the middle.

Thus, the overall aim was to reveal distinct mechanisms of translational regulation, i. e. through cis regulatory elements, paired uORFs and potentially yet new mechanisms involving uORFs. I was responsible for studying the regulation of uORFs. With my undergrad peers, we generated luciferase reporter plasmids whose translation started from 5’ untranslated regions (UTR) of genes with uORFs, such as Gcn5 and Hri2, or their variants carrying start codon mutations in uORFs. We integrated the reporter gene fragments into the genome of wild-type (WT) or gcn2-deleted yeast in the process of yeast transformation. We then looked for clones with correct integration by PCR. The correct clones were grown under both under unstressed and amino acid starvation conditions (the latter induced by addition of 3-aminotrizole). Cells were collected and measured for luciferase activity by DualGo reagent (Promega). Thought our

experiment minor changes were made to help increase the growth of yeast, such as using 150 ml Erlenmeyer flasks for incubation and varying forms of agitation between intervals of further incubation.