Fission Yeast mRNA Translational Regulation During Stress

Cassandra Delich, Biology

Katsura Asano, Professor of Genetics, Department of Biology

Summer 2018

This summer, the purpose of our research was to understand how control of mRNA translation from stages during stress is important in gene regulation in human cancer cells. I was able to see how cellular signaling controls translation by modulating mRNA recruitment through cis regulatory elements, or using delayed re-initiation mechanisms involving upstream ORFs (specifically uORF). The regulatory mRNAs encode transcription factors that allow genome-wide transcriptional responses. Amino acid starvation activates Gen2 eIF2a kinases, and then the eIF2a phosphorylation causes slow growth through inhibition of protein synthesis, which facilitates translation of hundreds of specific mRNA species. Cancer cells show resistance to metabolic stress, which causes them to establish themselves during their migration and metastasis from the out of control growth.

The model organism we used was a fission yeast, Schizosaccharomyces pombe. This type of yeast is very similar to human cells, as it has hundreds of candidate genes whose translation is stimulated by amino acid starvation with dependence to Gen2. Our goal this summer was to reveal distinct mechanisms of translational regulation through cis regulatory elements, paired uORFs, and potentially new mechanisms involving uORFs. I was responsible for studying the nucleotide motifs such as UGA(C/A)G. With my other undergraduate peers, I generated luciferase reporter plasmids whose translation starts from 5' untranslated regions (UTR) of genes with these motifs, and those deleted for the motifs. We integrated the reporter gene fragments into the genome of wild-type (WT) or Gen2-deleted yeast in the process of yeast transformation. Then we looked look for clones with correct integration by PCR. The correct clones were grown under both unstressed and amino acid starvation conditions. Cells were collected and measured for luciferase activity by the DualGo reagent (Promega).