Fall 2023

Using CRISPR to Generate Integrated Ssa4-GFP Reporter Strains

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Proteins play critical roles in numerous cellular processes. In order to synthesize these important molecules in eukaryotes, DNA is first transcribed into an intermediate molecule, mRNA, in the nucleus. The export of mRNA from its origin in the nucleus to the site of protein production, the cytoplasm, is an integral step in protein synthesis. When a cell is subjected to stress, such as heat shock, most mRNA export is halted, as export proteins are inactivated, and cellular machinery is redirected towards recovery. However, particular transcripts, including the Ssa4 transcript, are selectively exported for induced expression of the Ssa4 protein. The mechanism by which Ssa4 is exported is largely unknown. Therefore, the goal of this semester has been to insert GFP sequence into the endogenous genetic locus encoding for Ssa4 to make a series of strains that report on the selective export of the Ssa4 transcript. Using a plasmid that I previously generated that contains gRNA sequences for Ssa4 and PCR to generate GFP templates for HR-directed repair, I was able to successfully generate several reporter strains. These were initially phenotyped by fluorescent microscopy and have been confirmed by PCR. Future experimentation will aim to investigate the conditions that allow for selective Ssa4 export using the Ssa4-GFP fusions generated this semester.

Keywords: *Saccharomyces cerevisiae*, mRNA export, nuclear pore complex, Ssa4, CRISPR, GFP, HR repair