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Generating Improved RNA Localization Reporters Using the U1hp-U1A System

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Messenger mRNA (mRNA) is genetic material that is used to bridge between DNA in the nucleus and protein-making machinery in the cytoplasm of eukaryotic cells. The processes of transcription and translation are physically separated, so the localization of mRNA dictates whether the encoded protein can be generated. In order to observe the localization of specific transcripts in response to stimuli, tools are needed to track location of mRNA. For example, in response to heat shock and ethanol stress, most mRNA is retained in the nucleus while select transcripts are permitted to export, allowing the cell to drastically reprogram its gene expression. **The goal of this project is to develop an updated method to track the location of specific mRNA in live cells by building off previous tools**. In this approach, the transcript of interest is engineered to include the mRNA hairpins found in the U1A snRNA (U1hp), and green fluorescent protein (GFP) is fused to the RNA binding domain of the U1A protein that binds U1hp with high affinity. While previous studies have demonstrated the utility of this approach, plasmid-based expression of the U1A-GFP reporter results in variable expression among cells, limiting its utility and reproducibility. In order to limit the amount of variability in the production of the reporter protein, I have cloned the U1A-GFP protein into an integrative plasmid with promoters of different strengths. This allows for even expression of the reporter among all cells in a population. This collection of integrative plasmids have been generated by a step-wise cloning process. We are currently performing experiments to test whether selective mRNA export can be recapitulated using this updated reporter. The tests will show how accurate the reporter is at localizing mRNA within a cell under different stress conditions.