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Research Project Title: Gene drive inhibition by the anti-CRISPR proteins AcrIIA2 and AcrIIA4 in budding yeast

Although relatively new to the scientific field, the CRISPR-Cas system has become central to many research investigations in biology, medicine, biotechnology, and more. One such application has been applying this gene editing system, which originated in prokaryotes, to eukaryotic organisms. In this way, the ability of the Cas9 nuclease to induce a double stranded break in DNA has proven a useful tool for possible treatment or curing of genetic diseases. Additionally, some have used the CRISPR system in what is called a "gene drive": instead of acting in a haploid, the system is applied to a diploid cell. Then, once provided with a single-stranded guide RNA (sgRNA) and target in the wild-type DNA sequence, Cas9 creates a double stranded break. The gene drive cassette can contain a modified or deleted gene, and when the DNA attempts to repair itself, the gene drive cassette takes the place of the wild type gene and copies itself. Because of this, the modified gene is ensured to be spread across the population due to Super Mendelian genetics. Thus, the gene drive has the potential to control population growth, for example, in malaria-carrying mosquitoes. However, it is critical that further research is done on this technology including how to regulate and inhibit it. Recently, two "anti-CRISPR proteins," AcrIIA2 and AcrIIA4, were discovered in bacteriophages that inhibit the enzyme function of Cas9. The Finnigan lab uses budding yeast as a model system to study CRISPR-Cas9 gene editing and gene drives. My project included studying whether the AcrIIA2 and AcrIIA4 proteins could inhibit an active gene drive system. First, I cloned both anti-CRISPR genes as fusions to GFP to test whether they could be expressed in yeast cells and inhibit CRISPR editing—both proteins were able to disrupt the nuclease function of Cas9 including in a gene drive. However, adding the GFP onto the AcrIIA2 protein disrupted its function compared to the untagged version. Second, I tested small deletions and individual mutations to both anti-CRISPR proteins to study which amino acids were important for its inhibitory function and found several positions important for its ability to inhibit Cas9. Third, I built a yeast strain that with a Cas9 gene drive that also contained the AcrIIA2/A4 gene as well. The anti-CRISPR protein was regulated by a different promoter than Cas9—this allowed gene drive activity to be changed to various levels. Together this study demonstrated a new way to inhibit or alter CRISPR-based gene drives in living cells.