Mutating the Dsn1 Protein to Mimic Structural Phosphorylation

Madison Rose, Ann Vu, Rylee Evanger, Jack Vincent

The kinetochore, a multi-protein complex that attaches to DNA, helps to direct chromosome segregation during anaphase. Microtubules that are attached to the kinetochore pull the chromosomes to opposite poles. This attachment is important because kinetochore malfunction can result in missegregation of chromosomes. The Dsn1 protein is a part of the MIND protein subcomplex within the kinetochore. This protein affects the cell cycle progression and helps to correct chromosome alignment during mitosis. Kinetochore proteins have been found to have phosphorylation sites that help with microtubule attachment. The impact of phosphorylation on kinetochore function is not fully understood. The Sue Biggins lab at the Fred Hutch Cancer Center has found phosphorylation sites within the Dsn1 protein using mass spectrometry at serines 546, 547, and 554. We are examining the phenotypic consequence of phosphorylation on the Dsn1 protein by introducing mutations in the *DSN1* gene at these codons to change them to aspartic acid codons. The negative charge on aspartic acid may mimic structural phosphorylation. We will make these mutations using the CRISPR-Cas9 system, which creates a double stranded break near the codons we intend to modify, and a homology directed repair process to change the underlying sequence. We were able to successfully create our CRISPR vector with a guide RNA that will target breaks to the *DSN1* gene. We also successfully transformed that vector into yeast. We are currently confirming the presence of mutations using Sanger sequencing. Once confirmed, we will examine cell division phenotypes on the resulting mutants.