Utilizing the CRISPR-Cas9 system to investigate the chromosomal segregation effects of phosphonull mutations in *DSN1* in budding yeast

Kaylie Manix

The kinetochore is a multi-protein complex that mediates chromosomal attachments to microtubules during mitosis. Dysfunction in kinetochore regulation leads to aneuploidy in cells. Budding yeast are used to investigate kinetochore dysfunction because of the high level of conservation between budding yeast and humans. Dsn1, a kinetochore protein, is part of the MIND protein complex; the MIND complex contributes to microtubule attachment and produces pulling forces by depolymerization. Phosphorylation of Dsn1p is part of the MIND complex regulation. Therefore, phosphorylation sites within Dsn1 should have an effect on the MIND complex functionality. Through mass spectrometry analysis, the Sue Biggins lab at Fred Hutchinson Cancer Center identified three serines in Dsn1p 546, 547, and 554 as phosphorylation sites. Utilizing the CRISPR-Cas9 system and homology-directed repair (HDR) the serines were changed to alanines in budding yeast to create a version of Dsn1 that cannot be phosphorylated at 546, 547, and 554, *dsn1-S546A/S547A/S554A*. Polymerase chain reaction (PCR) of genomic DNA (gDNA) harvested from successfully transformed colonies were sent out for Sanger sequencing to determine successful transformation. Sanger sequencing results are pending. Phenotype analysis of the confirmed *dsn1-S546A/S547A/S554A* mutant strains is next to determine the effect of phosphorylation prevention on budding yeast.