CRISPR-Cas9 Mutagenesis of Phosphorylation Sites 380 and 386 in the Kinetochore Protein Dsn1

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CRISPR-Cas9 Mutagenesis of Phosphorylation Sites 380 and 386 in the Kinetochore Protein Dsn1The interaction between the kinetochore and spindle microtubule serves as a checkpoint during the transition from metaphase to anaphase in the cell cycle. Bipolar microtubule attachment and tension sensing is required for successful segregation of sister chromatids, and progression through the cell cycle. Incorrect attachment will lead to cells containing excess genetic material, or not enough; both of which will compromise the cell's survival. Proteins that make up the kinetochore, such as Dsn1, are still being investigated for their role in chromosome segregation. Dsn1 is a protein located in the MIS/MIND complex, a component of the outer kinetochore, and bridges kinetochore subcomplexes involved in microtubule attachment and tension sensing.

Phosphorylation is known to alter the structure and function of proteins. We were interested in whether these phosphorylation events impacted the function of Dsn1 within the kinetochore. To test this, we aimed to mutate the *DSN1* gene at codons that code for amino acids known to be phosphorylated. Specifically, we targeted two threonine amino acids at sites where mass spectrometry analysis has confirmed Dsn1 is phosphorylated: T380 and T386.

We transformed yeast with a vector expressing the CRISPR-Cas9 system engineered to target a DNA break in the *DSN1* gene, and a homology directed repair DNA molecule (HDR) that would induce mutations changing the target codons to valine (T380V and T386V) during the repair of the break. After obtaining yeast that were transformed with these DNAs, we then amplified this region through a PCR reaction and sent out the DNA for Sanger sequencing to confirm the presence of our intended mutations. The integration of the *dsn1-T380/386V* mutations was successful. Phenotypic testing of mutated yeast, including temperature sensitivity and benomyl sensitivity, will begin to show the role of phosphorylation of these sites in the function of Dsn1.