

# **Investigating CRISPR-Induced Mutations Affecting Chromosomal Segregation in the Dsn1 Outer Kinetochore Protein**

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Mitosis is a tightly regulated cellular process fundamental to the growth and development of eukaryotic organisms. Improper segregation of chromatids results in aneuploidy where resulting daughter cells contain abnormal numbers of chromosomes. The kinetochore, a hierarchical collection of protein complexes, is instrumental in proper segregation as it forms on centromeric DNA during mitosis and mediates connections between chromatids and microtubules. Establishing appropriate connections is an intrinsically error prone step and requires cell-cycle checkpoints to arrest division when errors occur. Chromatids achieve proper biorientation and progress to anaphase only when the kinetochore experiences proper tension. In budding yeast, absence of tension initiates the Ipl1 kinase to phosphorylate kinetochore structures, such as the Dsn1 outerprotein, halting cell cycle progression. Influence of phosphorylation upon the outerprotein responsible for microtubule-binding activity is not fully understood. Our collaborators found evidence of phosphorylation at loci 546, 547 & 554 within Dsn1 via mass spectrometry. Our goal is to better understand phenotypic implications of Dsn1's phosphorylation by introducing phospho-mimetic and phospho-null missense mutations at targeted phosphorylation sites through the use of the CRISPR-Cas9 system, mimicking states of both persistent and absent phosphorylation. Molecular biology techniques were used in introducing and propagating mutations in budding yeast which were then subjected to phenotypic testing, such as exposure to varying concentrations of a microtubule depolymerizing drug to reveal growth defects resulting from these missense mutations. Double mutants at loci 546 and 547 were successfully created for both mimetic and null variants. Preliminary phenotypic tests reveal no distinction between control and mutants suggesting phosphorylation at these sites alone is not sufficient for proper function of Dsn1.