Mutating the Dsn1 Protein to Mimic Structural Phosphorylation

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The kinetochore is a complex of proteins that attaches to the centromere and to the spindle microtubules which segregates copies of DNA to the opposite poles during mitosis. Kinetochore attachment and assembly during chromosome segregation is important for cellular development. The malfunction of kinetochores can lead to the missegregation of the DNA leading to abnormal numbers of chromosomes. Dsn1p is a component of the outer kinetochore MIND complex that affects the cell cycle progression and is required for the correct alignment and segregation of chromosomes. Our collaborators have found that Dsn1p is phosphorylated at various amino sites including serine 546, 547, and 554 positions in the protein through mass spectrometry analysis. Phosphorylation is used as a mechanism to regulate protein function and transmit signals through a cell. In this study we aim to use budding yeast to measure the potential impact of Dsn1p phosphorylation by mutating codon sites S546, S547, and S554 in the *DSN1* gene. We will use the CRISPR-cas9 system to make mutations at these positions that mimic constant phosphorylation. Our work is still in progress, but our sequencing analysis has shown that we successfully cloned the guide RNA encoding sequence into the CRISPR vector. We are looking forward to transforming our CRISPR vector into yeast to create our phospho-mimetic mutations.