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

TBIOMD 495 | Briann Jolene Santos, Yasmine Silva & Dr. Jack Vincent

Background

One of the many protein complexes essential in ensuring proper separation of DNA copies is known as the kinetochore, which mediates connections between DNA and spindle microtubules.

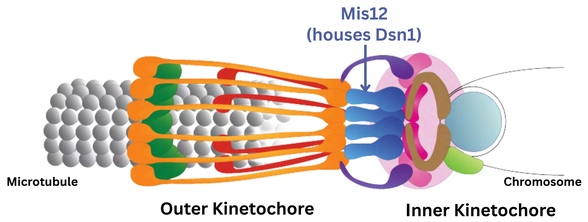


Figure 1. Model of kinetochore structure. Image credit to the Biggins laboratory at the Fred Hutch Cancer Center.

Rather than microtubules attaching to the centromere, they attach to kinetochores. It is within this connective step of mitosis where the tension & pulling force is provided to separate duplicate DNA molecules (sister chromatids) to opposite poles of the cell.

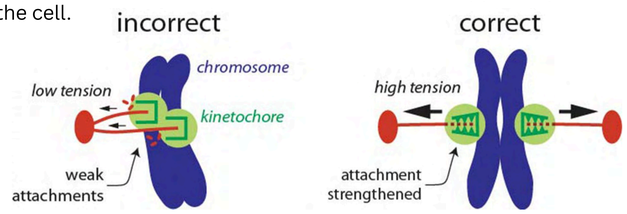


Figure 2. Depicts an example of incorrect, unstable microtubule-kinetochore connections (both kinetochores bound to microtubules emitting from same pole) which generates low tension at the kinetochore. Correct, bi oriented attachments occur when each kinetochore is bound to dynamic microtubules from opposite poles, creating high tension. Image credit to the Asbury Lab at the University of Washington.

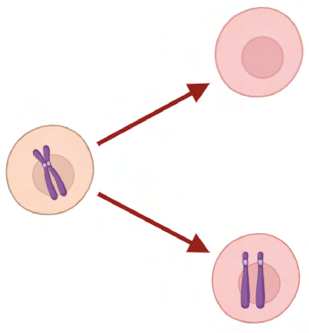


Figure 3. Aneuploidy is the result of improper chromosomal segregation where daughter cells produced contain abnormal/unequal number of chromosomes.

Mistakes in this crucial step results in aneuploidy, where replicated daughter cells inherit an abnormal number of chromosomes. This outcome can be very detrimental to subsequent cellular function and division. Errors in the binding step often are caused by destabilized microtubule-kinetochore connections lacking the necessary tension which in turn initiates the phosphorylation of specific residues within kinetochore proteins from the conserved Ipl1 protein kinase, allowing for the cell's redemption to achieving proper microtubule attachment alignment in the cell (Biggins 2013).

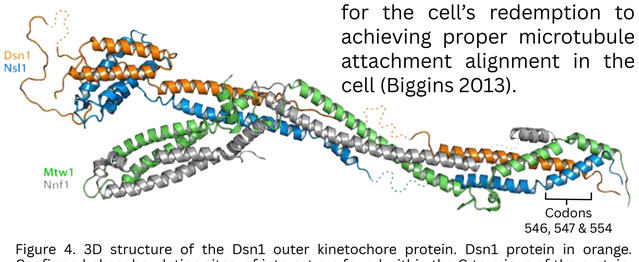
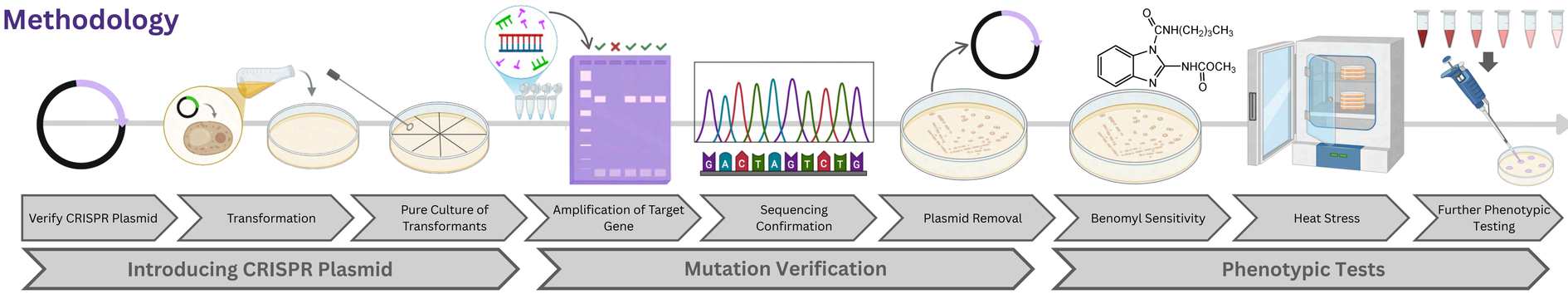


Figure 4. 3D structure of the Dsn1 outer kinetochore protein. Dsn1 protein in orange. Confirmed phosphorylation sites of interest are found within the C-terminus of the protein. Target loci, codons 546 and 547 are found within alpha helix while 554 is found within an unstructured area (Dimitrova et al. 2016).

Methodology



Results

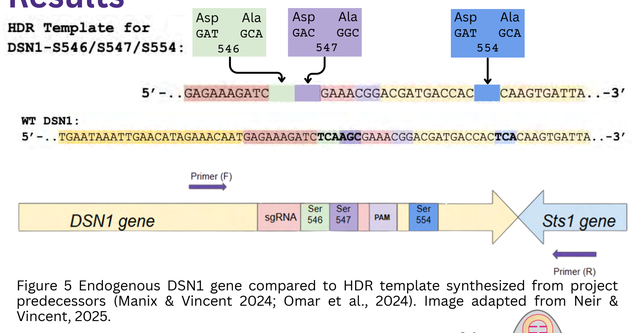


Figure 5 Endogenous DSN1 gene compared to HDR template synthesized from project predecessors (Manix & Vincent 2024; Omar et al., 2024). Image adapted from Neir & Vincent, 2025.

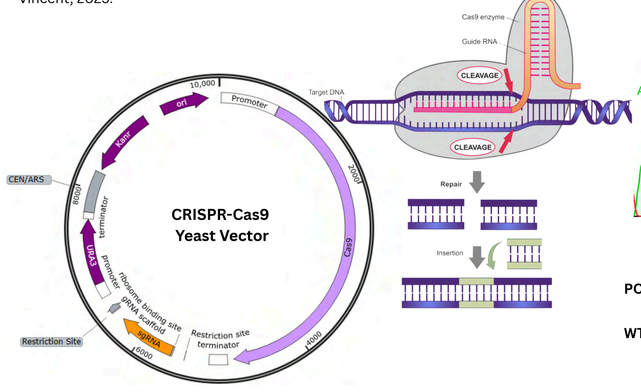


Figure 6. CRISPR plasmid designed by from Manix & Vincent 2024; Omar et al., 2024. Visual depicting the encoded cas9 enzyme, guide rna, and visual of homology-directed repair (HDR).

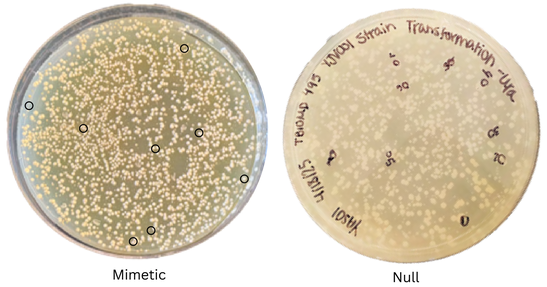


Figure 7. Selected transformed yeast colonies streaked onto -URA media allowing only plasmid-containing colonies to grow. Selected colonies will be analyzed for presence of mutations via PCR and sequencing.

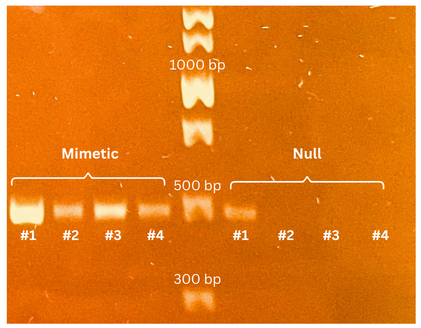


Figure 8. Gel electrophoresis of a ~500 bp portion of Dsn1 gene amplified by PCR.

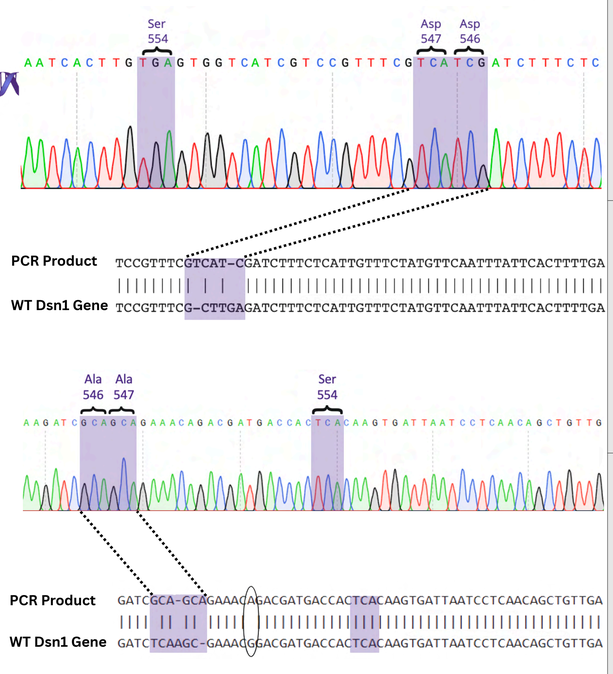


Figure 9. Sequencing data confirms successful creation of double mutant at loci 546 and 547 loci for both mimetic and null variants. Residue 554 remained unchanged for both variants. An unintended silent point mutation was introduced into null variant at residue 549.

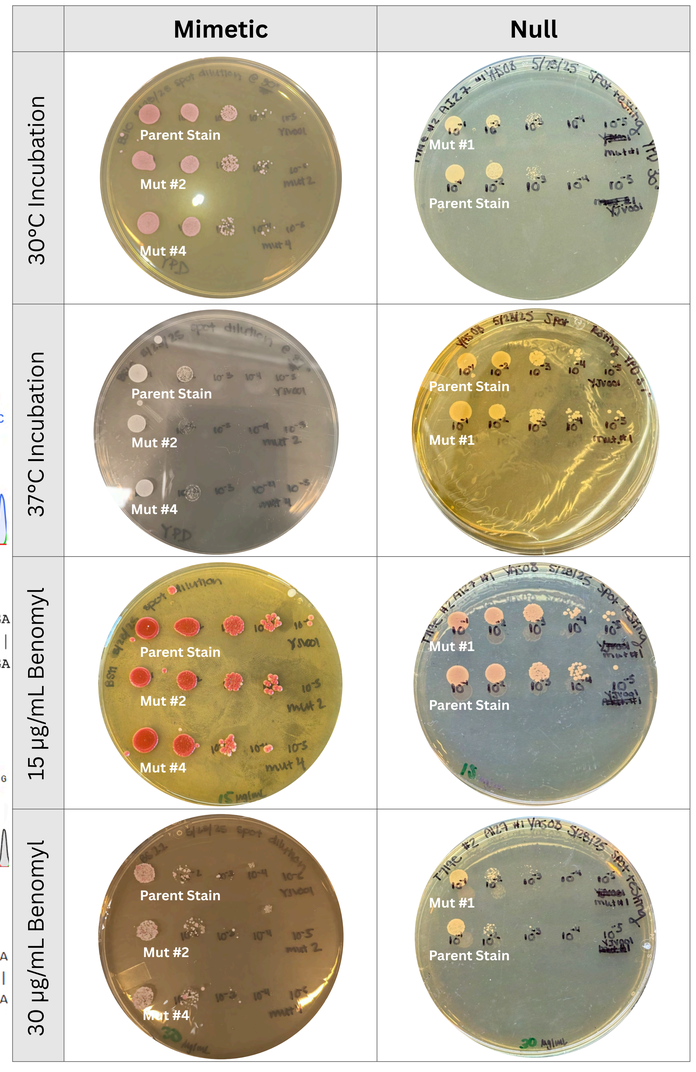


Figure 10. Heat stress and varied benomyl concentration phenotypic tests. Both mimetic and null variants presented no drastic difference compared to YJV001 parental strain.

Experimental Approach

Through mass spectrometry, the Biggins lab at the Fred Hutch Cancer Center found three phosphorylation sites in close proximity, serine loci 546, 547, & 554, within the Dsn1 outer protein which are our targeted sites. We aim to understand the potential role of phosphorylation at these sites by inducing triple mutations at the corresponding codons to either prevent or mimic phosphorylation. Through the introduction of these mutations, we'll have the capacity to investigate the influence phosphorylation of Dsn1 possesses in chromosomal segregation.

Conclusions

CRISPR plasmid was successfully integrated into yeast strain and double mutations at loci 546 and 547 were confirmed for both phospho-mimetic and phospho-null variants. Preliminary phenotypic tests revealed no major distinction from parental strain for both mimetic and null mutation variants suggesting that phosphorylation at loci 546 and 547 alone isn't sufficient enough to impact proper function of Dsn1 during mitosis.

Future Direction

Based on experimental evidence gathered from sequencing and preliminary phenotypic tests, future work should aim to successfully producing a triple mutant to further investigate the influence of a phosphorylated state of Dsn1 and the implications this may yield in achieving appropriate microtubule-kinetochore attachments during mitosis. If phenotypic differences are observed, subsequent studies should incorporate more quantitative approaches to assess cell growth, such as identifying potential cell cycle stalling at specific checkpoints.

Acknowledgments

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References