Phosphorylation at MELT Domains and it's Importance for STU1 Function

Chromosomal segregation is an essential function in ensuring that cells accurately inherit chromosomes during cell division. The segregation of chromosomes is led by kinetochore-microtubule attachments in which the chromosomes are properly aligned by the kinetochores and, once aligned, pulled apart by the microtubules. In order to further understand the function and behavior of proteins in kinetochores, budding yeast serves as the organism of study due to its simplicity in kinetochore structure and conservation of its functions and sub complexes in eukaryotic organisms. Stu1p, a component of the budding yeast mitotic spindle, is essential for growth and is known to be well conserved in the MELT domain, an area of protein that has been demonstrated to be phosphorylated. However, is phosphorylation at these domains essential to Stu1p function? In order to test its importance, CRISPR was used to create a mutation at the codon that codes for Threonine to a Valine at the 1034 position, which would prevent phosphorylation. We began by designing a guide RNA and repair template which would be cloned and used in the CRISPR vector. We then did a Gibson assembly to insert our guide RNA into the CRISPR vector and confirmed our success through DNA sequencing. Finally, we introduced the CRISPR vector into budding yeast and performed initial testing on phenotype. Although we have yet to confirm whether or not the CRISPR system was successful in making a mutation in our budding yeast, we can confirm colony growth of various sizes. Sequencing to confirm transformation followed by further phenotype analysis are the next steps in further development of this research.