The Role of Phosphorylation in the SK-rich and C-terminal Region of the Kinetochore-Associated Stu2 Protein in Budding Yeast

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Kinetochores are specialized protein complexes that connect spindle microtubules and segregate chromosomes during cell division. In order to properly achieve chromosome segregation, kinetochores are subject to checkpoint pathways that ensure proper inheritance of copied DNA. These checkpoint pathways monitor the attachment of microtubules to kinetochores or the tension or lack thereof that is exerted on kinetochores. Checkpoints are enacted through phosphorylation of proteins that control cell division. Stu2 is a kinetochore-associated protein in the model organism S. cerevisiae (budding yeast) which can either polymerize or destabilize microtubules in response to changing tensions during mitosis. The Stu2 protein contains numerous phosphorylation sites within its SK and C-terminal regions which have been previously identified through mass spectrometry. In order to test the importance of these Stu2 protein phosphorylation sites, we induced mutations which incorporate non-phosphorylating amino acids at these sites. We used MegaWHOP cloning to combine previously constructed mutations in both the SK-rich and C-terminal domains as well as introduce a new mutation not covered by previous efforts. The next steps will be to introduce this modified *stu2* gene into yeast to determine whether these mutations result in impaired cell viability or a delay in cell cycle progression due to chromosome segregation defects. We are currently waiting on sequence confirmation of our mutagenized *stu2* in order to determine if all mutations are present within the newly made plasmid.