Analysis of Ipl1 Kinase Phosphorylation Sites on the Kinetochore-Associated Protein Stu2 Through Mutagenesis

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Aneuploidy is the missegregation of chromosomes that leads to genetic disorders such as Down syndrome, mosaic variegated aneuploidy, and cancer. One of the main causes of aneuploidy is the improper attachments made between kinetochores and microtubules during mitosis. Kinetochores are protein structures that play a significant role in biorientation of chromosomes and serves as attachment points for microtubules in chromosome segregation. Other proteins collaborate with kinetochores to ensure proper connections are made, strong connections are stabilized, and weaker connections are destabilized to delay chromosome segregation. Previous studies have found evidence that the Stu2 protein and Ipl1 kinase in the model organism *Saccharomyces cerevisiae* have similar functions in regulating tension between kinetochores and microtubules. This study aimed to determine if the function of Stu2 is dependent on phosphorylation by Ipl1 kinase by creating mutations at Ipl1 target sites on the *STU2* gene and observing phenotypic consequences in *S. cerevisiae*. A total of five Ipl1 target serine codons on *STU2* were identified as possible mutation sites. Utilizing the megaprimer whole plasmid (MEGAWHOP) cloning technique, additional mutations were made to a pSB2232 variant that previously contained *stu2-S430A* and *stu2-S593A* mutations.