Phosphorylation of T1080 mediates the interaction of kinetochore proteins Stu1 and Slk19

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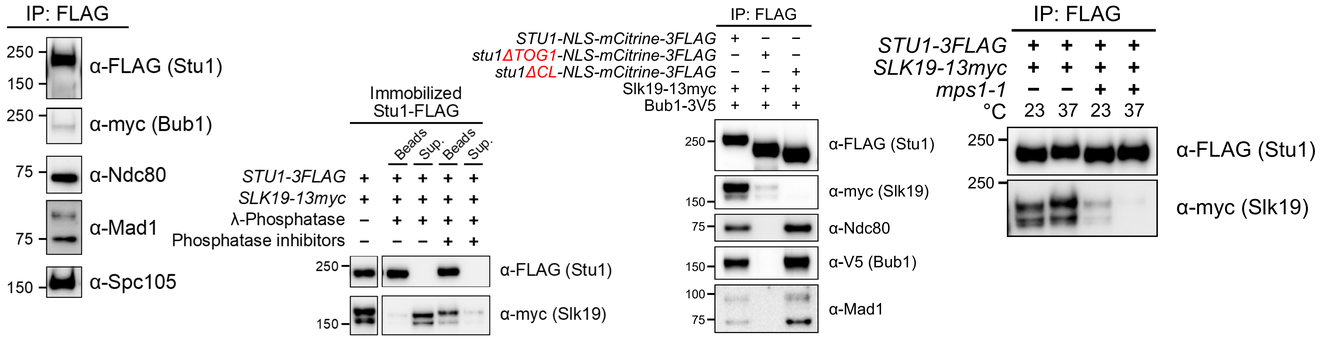
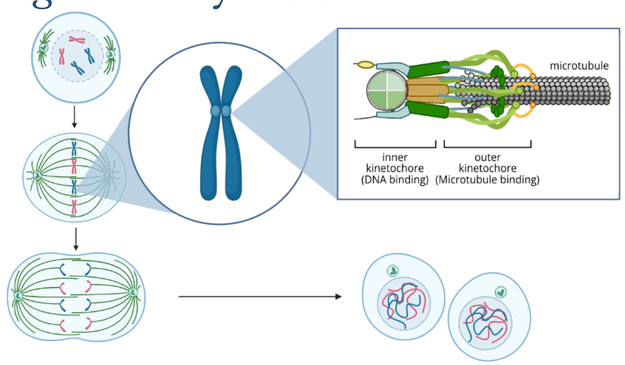
Abstract

When cells divide, they undergo mitosis to equally distribute replicated chromosomes between their daughter cells. Essential to this process is the kinetochore, a megadalton protein complex that attaches the sister chromatids to the mitotic spindle. Improper or failed attachments may result in aneuploidy, the uneven distribution of chromosomes between daughter cells, which is a hallmark of cancer cells. In *Saccharomyces cerevisiae*, unattached kinetochores recruit Spindle Assembly Checkpoint (SAC) complexes through Mps1 kinase activity, which prevents progression into anaphase until all kinetochores have formed proper attachments to microtubules. Mps1 also recruits Stu1 and Slk19 to unattached kinetochores to enable timely kinetochore capture by microtubules through kinetochore clustering. Here, we investigate how Mps1 controls kinetochore clustering through the phosphorylation of Stu1's CL region. We find that a Stu1 mutant that disrupts Mps1 phosphorylation abrogates Stu1-Slk19 interaction but does not affect kinetochore clustering, suggesting a more complex relationship between Stu1, Slk19, and kinetochore clustering.

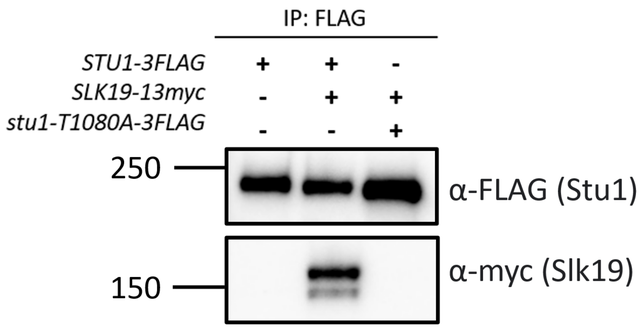
Stu1-Slk19 interaction is dependent on Mps1 kinase activity

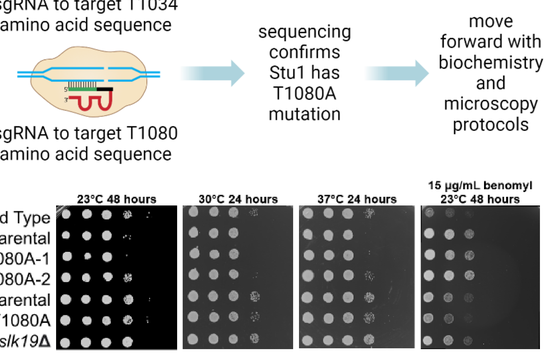


The kinetochore is essential for high-fidelity mitosis

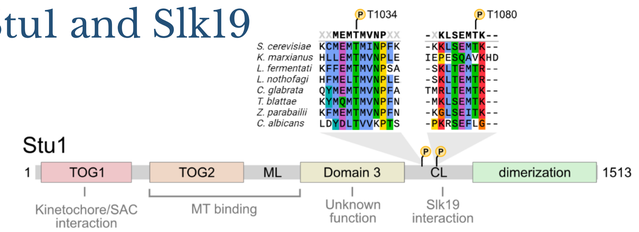


T1080A destroys Stu1-Slk19 interaction

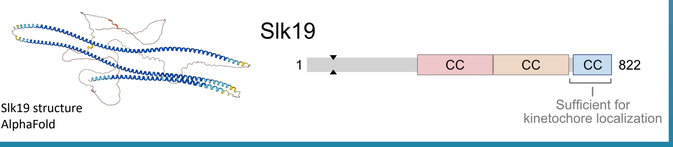




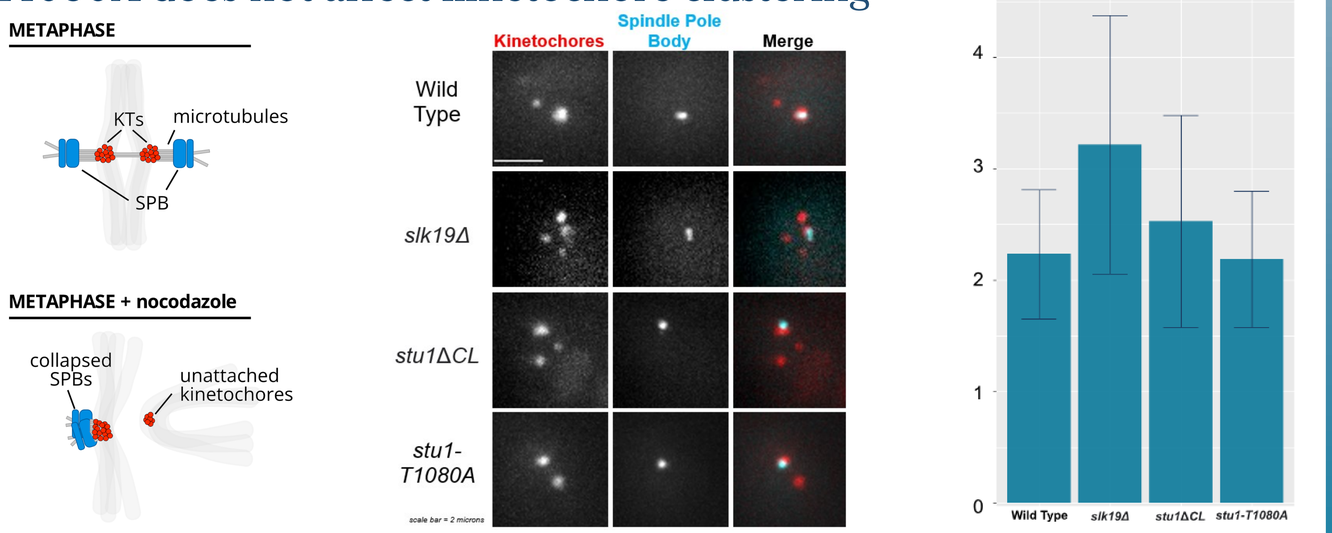
Stu1 and Slk19



Stu1	Slk19
•Essential gene •Microtubule "rescue factor" •Binds and cross-links the microtubules to stabilize the spindle	•Involved in mitotic progression through anaphase and completion of mitosis •Helps stabilize spindle microtubules •Involved in unattached kinetochore clustering



T1080A does not affect kinetochore clustering



Conclusions

1. Phosphorylation of the 1080 Threonine in the CL domain of Stu1 by Mps1 is required for the interaction between Stu1 and Slk19
2. The mutation of the 1080 Threonine to Alanine does not appear to affect kinetochore clustering, suggesting a more complex interaction between Stu1, Slk19, and kinetochore clustering

Future Directions

Through site-directed mutagenesis, we hope to generate the T1034A mutant for use in biochemistry and microscopy experiments. By comparing the T1034A mutant to the T1080A mutant, we hope to gain a more nuanced understanding of the mechanism of interaction between Stu1 and Slk19 and how this interaction affects kinetochore clustering.

Acknowledgments

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