Investigating Spc24 Spatiotemporal Mitotic Function

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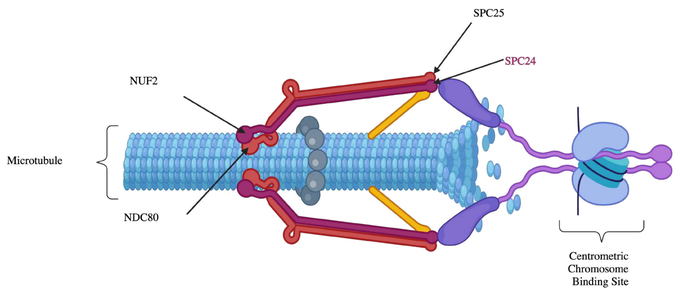
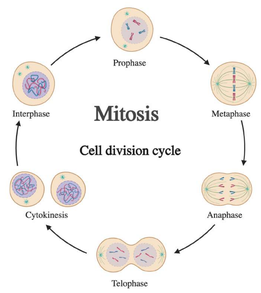
ABSTRACT

Mitosis has captivated cell biologists for centuries- its functioning and proper distribution of chromosomes into new cells is vital to all eukaryotic life and missteps or malfunctions have massive implications. There are thousands of genes and corresponding proteins involved in the process, a large number of which have not been fully studied nor understood as of yet- the NDC80 complex is one of them. As of yet, it's known that this complex is essential for correct chromosomal segregation through kinetochore-microtubule attachment. Overexpression of the NDC80 complex was found to induce tumor formation in a mouse model via activation of the mitotic checkpoint. To assess Spc24 localization, we transfected female rat kangaroo kidney epithelial (PTK1) cells, from the species Potorous tridactylus, with a plasmid containing the fluorescently tagged Spc24 protein. Using fluorescence microscopy, we measured the cytoplasm-normalized intensity of Spc24 localization at the kinetochores and centrosomes in each phase of mitosis. We also directly compared the Spc24 gene sequence in PTK1 to that of humans. We found that the intensity of Spc24 peaked at the kinetochores during anaphase and at the centrosomes during prometaphase. This highlights the NDC80 complex's role in microtubule binding to kinetochores, particularly in correctly partitioning chromatids into daughter cells. This work is significant in describing the specific spatiotemporal function Spc24 throughout mitosis.

INTRODUCTION

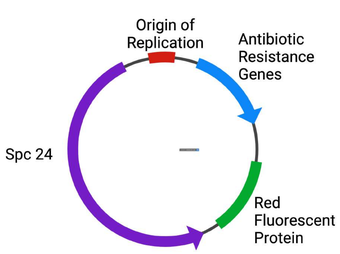


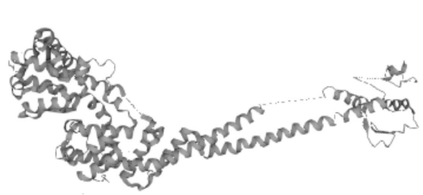


- Spc24 is part of the NDC80 complex, consisting of Hec1(NDC80), Nuf2, Spc24, and Spc25.
- NDC80 complex is essential for kinetochore-MT attachment as well as proper chromosome segregation
- NDC80 complex is regulated by Aurora B Kinase phosphorylation of Hec1

METHODS

- Construction of a mammalian expression vector containing PTK1-Spc24 tagged with mTagRFP-T and 3XFLAG, then transfected via transgene insertion into PTK1 cells
- Cells were fixed with DAPI, to fluorescently stain DNA & RFP tagged Spc24
- Fiji ImageJ was used to measure fluoresence intensity of Spc24 localized at kinetochores and centrosomes
- DNA was sequenced using ncbi blast and then mapped and analyzed via Benchling





NDC80 Complex composed of Spc24, Spc25, Nuf2, and Hec1

RESULTS

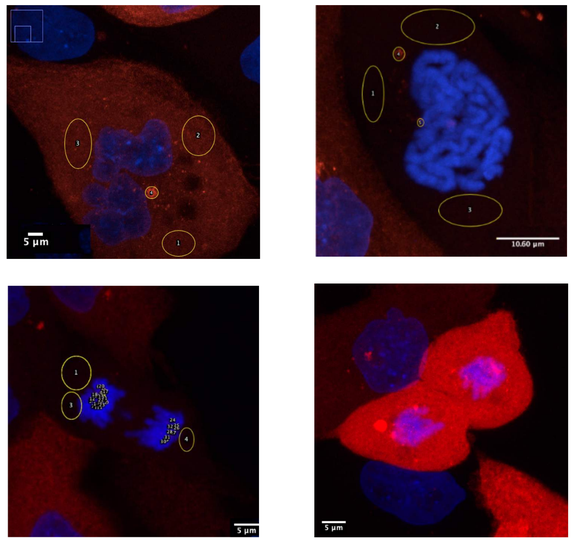


Figure 1. Images from confocal fluroescence microscopy. DNA is stained with DAPI, a blue fluorescing stain upon binding to AT regions of dsDNA. Spc24 was tagged with red fluorescent protein to show it's localization. The image on the upper left corresponds to a cell in prophase, the upper right in prometaphase, lower left in anaphase and the lower right in telophase. Fluoresence was measured within the areas circles in yellow, corresponding to either the cytoplasm, kinetochores, or centrosomes.

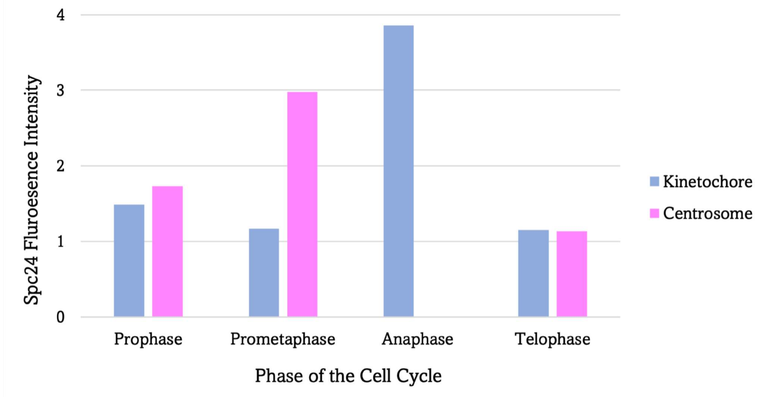


Figure 2. Spc24 fluroescence intensity localized at the kinetochores and the centrosomes, noramlized by Spc24 fluoresence in the cytoplasm. Measurements were made at each phase of mitosis

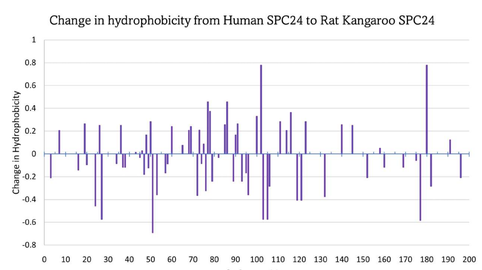


Figure 3. Change in amino acid hydrophobicity from Human to Rat Kangaroo SPC24. Amino acid differences between Human and Rat Kangaroo SPC24 sequences were mapped for differences in hydrophobiciity, with the changed Rat Kangaroo amino acid subtracted from the corresponsing Human amino acid. Codon position was included to see clusters of hydrophobicity changes.

CONCLUSIONS

- Spc24 was heavily localized towards the site for kinetochore-microtubule attachements
- Spc24 was also localizing to centrosomes, particularly during prometaphase
- These two conclusions could be due to Spc24's intrinsic microtubule binding capacity or it acting as part of the overall NDC80 complex
- Further studies are needed to confirm these findings

FUTURE DIRECTIONS

- Does Spc24 have unique microtubule binding properties? Or is it acting as part of the overall NDC80 complex?
- Mapping amino acid changes and doing more analysis of sequence data as well as Spc25 to observe changes in Spc24-Spc25 binding interface between humans and rat kangaroos
- Controlled knockdowns of each component of NDC80 (HEC1, NUF2, SPC24, SPC25)
- Aurora-B knockdown to observe phenotypical changes in affected cells to know more about the comprehensive function of Aurora-B kinase

REFERENCES

Meng, Q. C., Wang, H. C., Song, Z. L., Shan, Z. Z., Yuan, Z., Zheng, Q., & Huang, X. Y. (2015). Overexpression of NDC80 is correlated with prognosis of pancreatic cancer and regulates cell proliferation. American Journal of Cancer Research, 5(5), 1730. /pmc/articles/PMC4497439

Wei, R., Sorger, P., Harrison S. Molecular organization of the Ndc80 complex, an essential kinetochore component. PNAS (2005). https://doi.org/10.1073/pnas.0501168102

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