INVESTIGATING THE ROLE OF HCP-3 DURING CELL DIVISION IN THE NEMATODE *C. ELEGANS*

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Faculty Mentor: Dr. Joost Monen
Faculty Readers: Dr. Catalin Martin and Dr. Thomas Owen

Outline of Talk

1. Why is cell division important?
2. *C. elegans* makes an ideal organism to study cell division
3. CENP-A
4. Purpose of the study
5. Immunofluorescence Assay
6. Conclusions and Future Directions

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1. **Why is cell division important?**
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6. Live Imaging Assay
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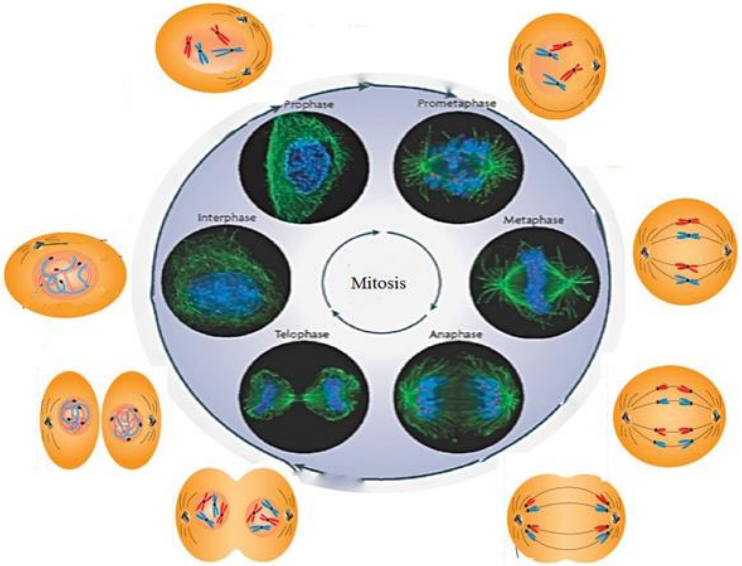
Why is cell division important?

All organisms undergo this process

Understanding at basic level translates to understanding in humans



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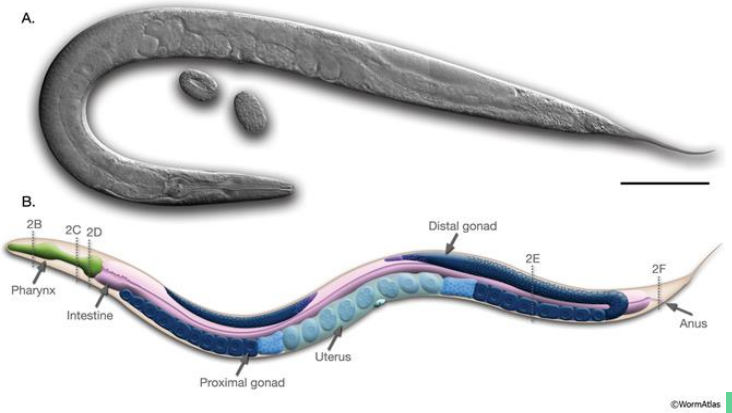
C. elegans makes an ideal organism to study cell division

- Genetic model system
- Cheap
- Transparent
- Embryos are HUGE
- **Great model to study cell division**



http://makeagif.com/gif/c-elegans-movement-g8AeNh

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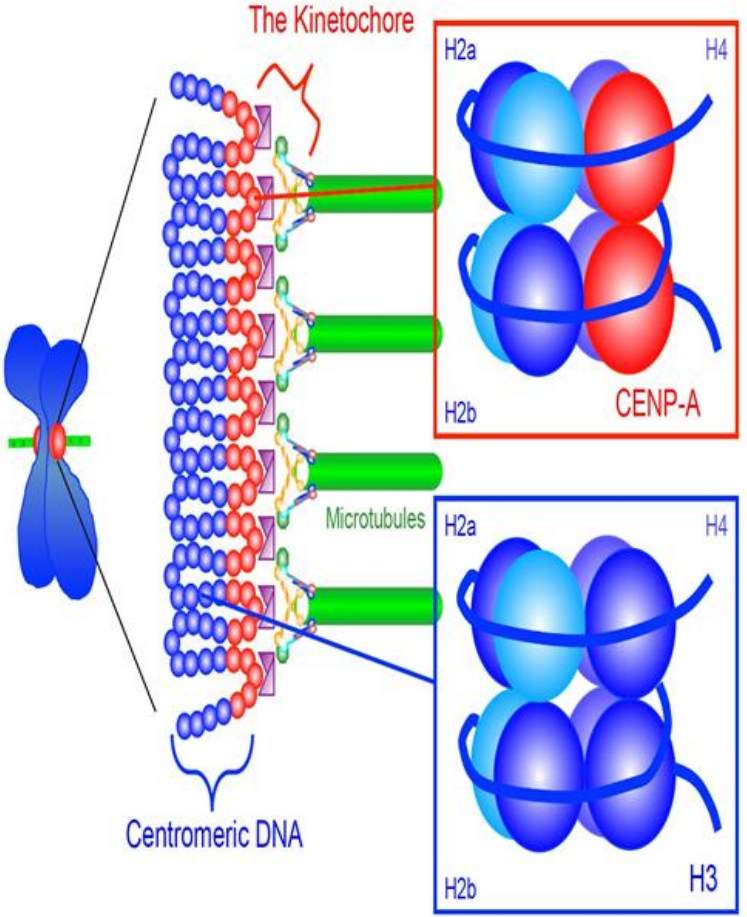
CENP-A

- Highly conserved Histone-H3 like protein
- Critical to centromere specificity and kinetochore assembly in all eukaryotes
- *C. elegans* are unique in that they have **2 CENP-A homologs**
  - HCP-3
  - CPAR-1



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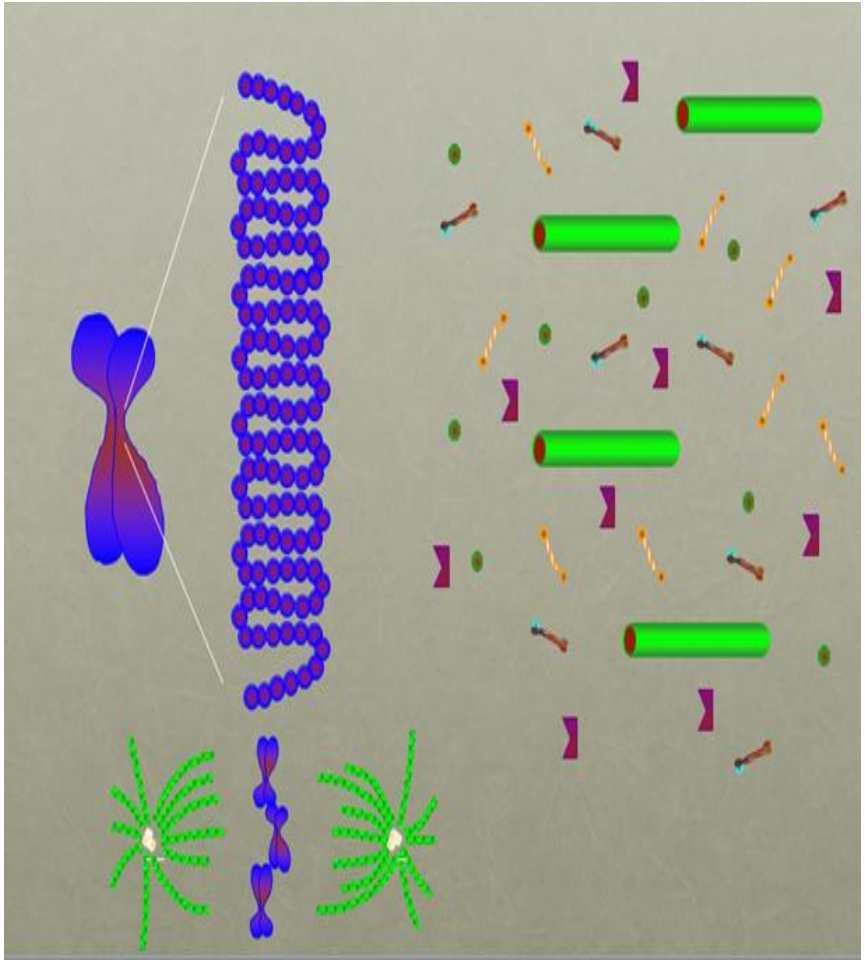

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Purpose of the study

- To investigate the divergent roles of HCP-3 and CPAR-1 in cell division in *C. elegans*
    - To develop and optimize assays to be able to characterize the differences
        - Determine localization of HCP-3
            - Immunofluorescence
        - Characterize the functional consequences of depleting HCP-3
            - RNAi

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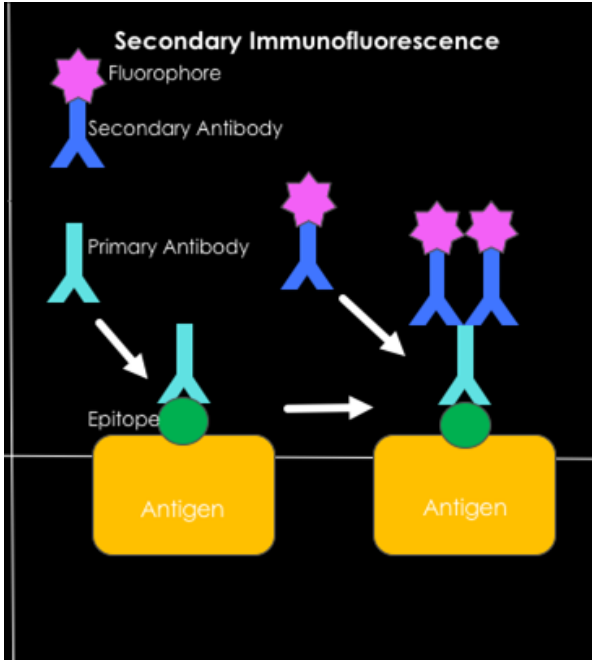
Immunofluorescence

Allows us to visualize chromosomes, microtubules, and the CENP-A homologs in the developing embryo



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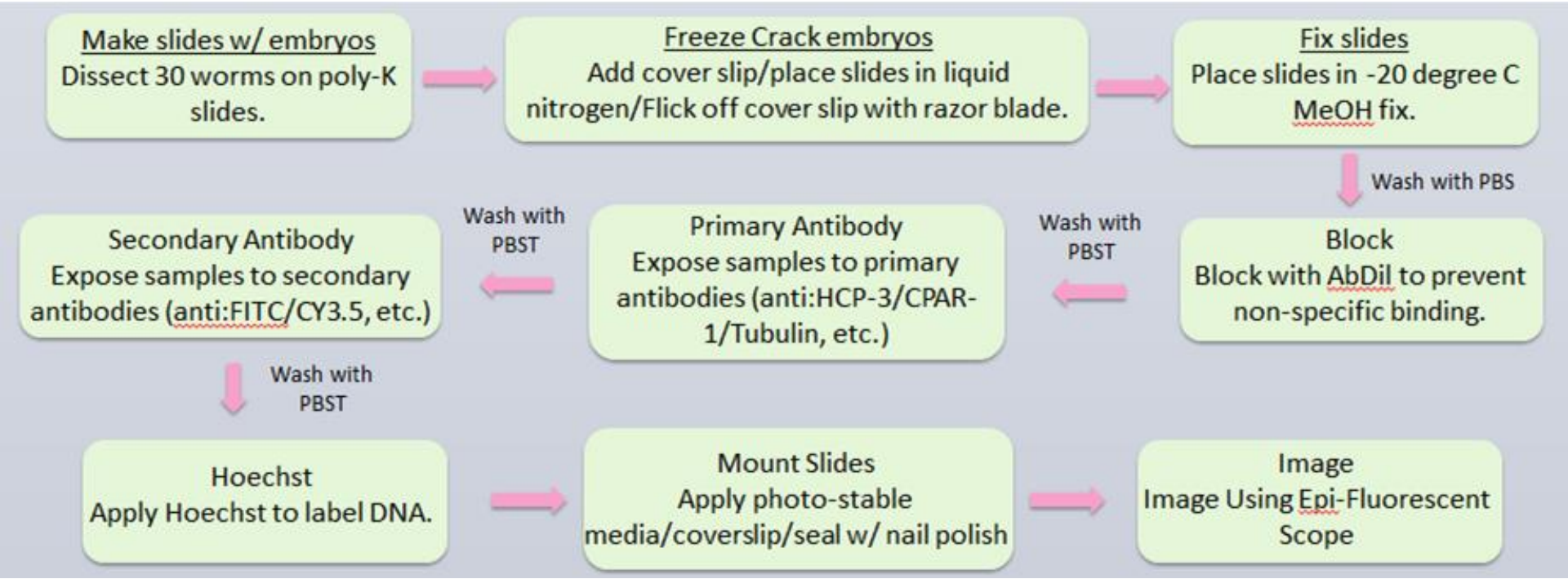
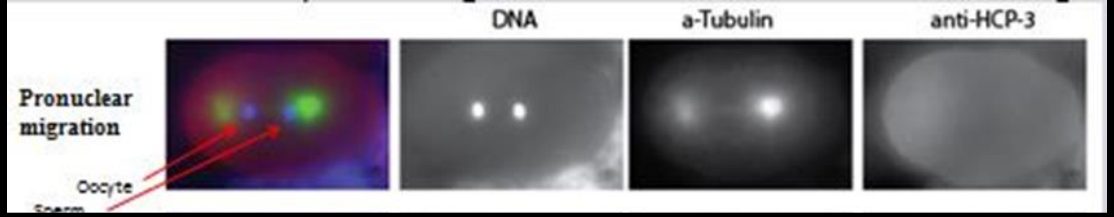
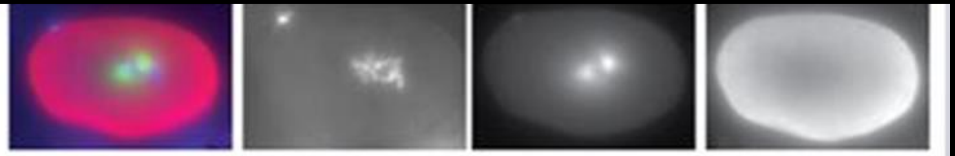
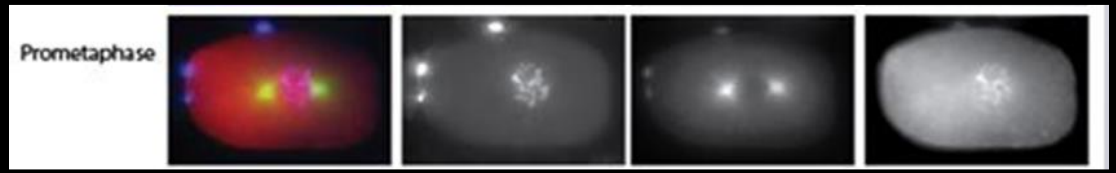
Immunofluorescence Methodology

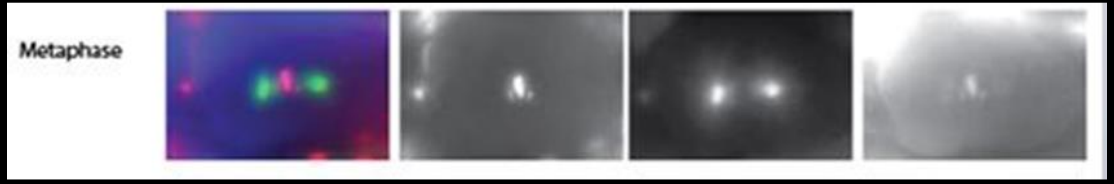



DNA
a-Tubulin
anti-HCP-3
Pronuclear migration
Oocyte

	DNA	a-Tubulin	anti-HCP-3
Prophase			

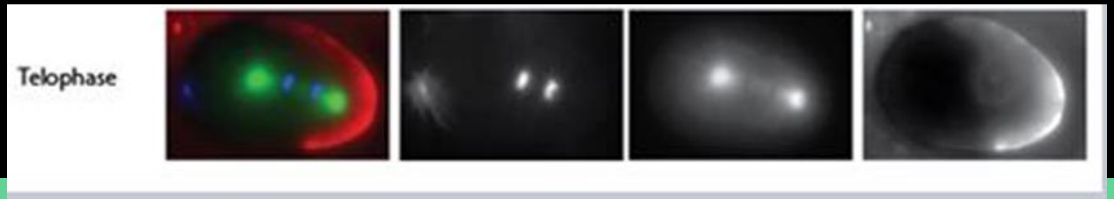
DNA a-Tubulin anti-HCP-3



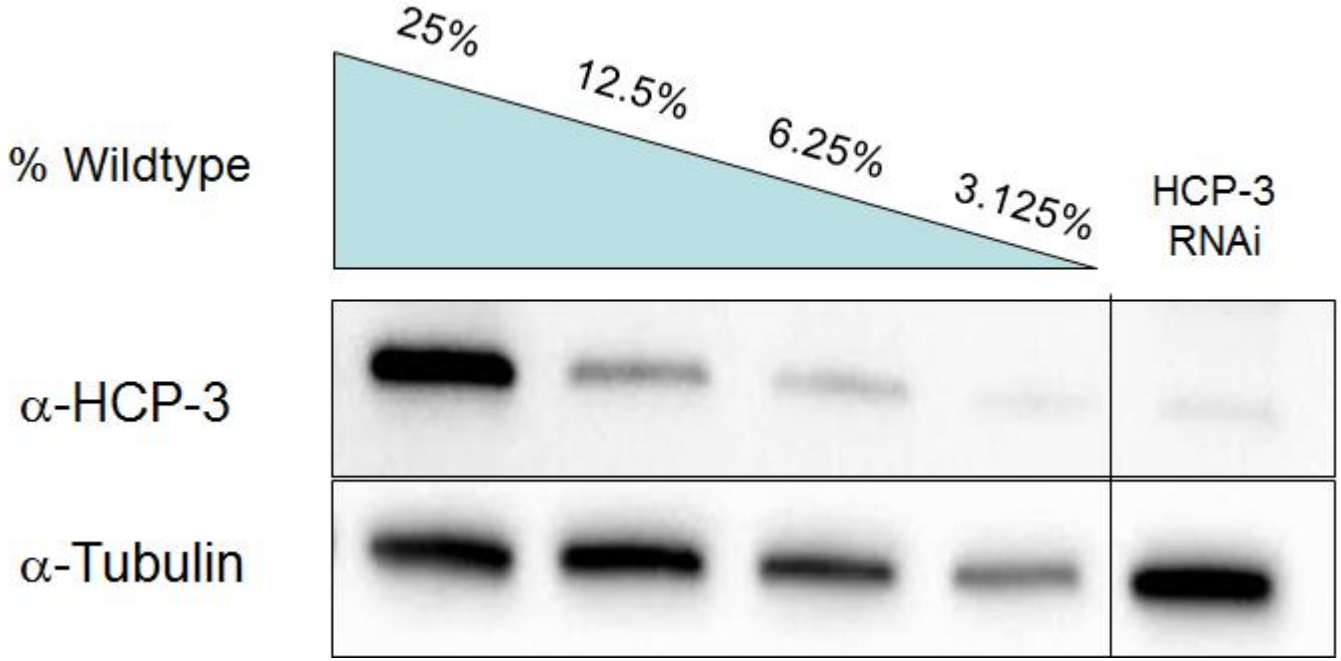

Metaphase

DNA a-Tubulin anti-HCP-3

DNA a-Tubulin anti-HCP-3



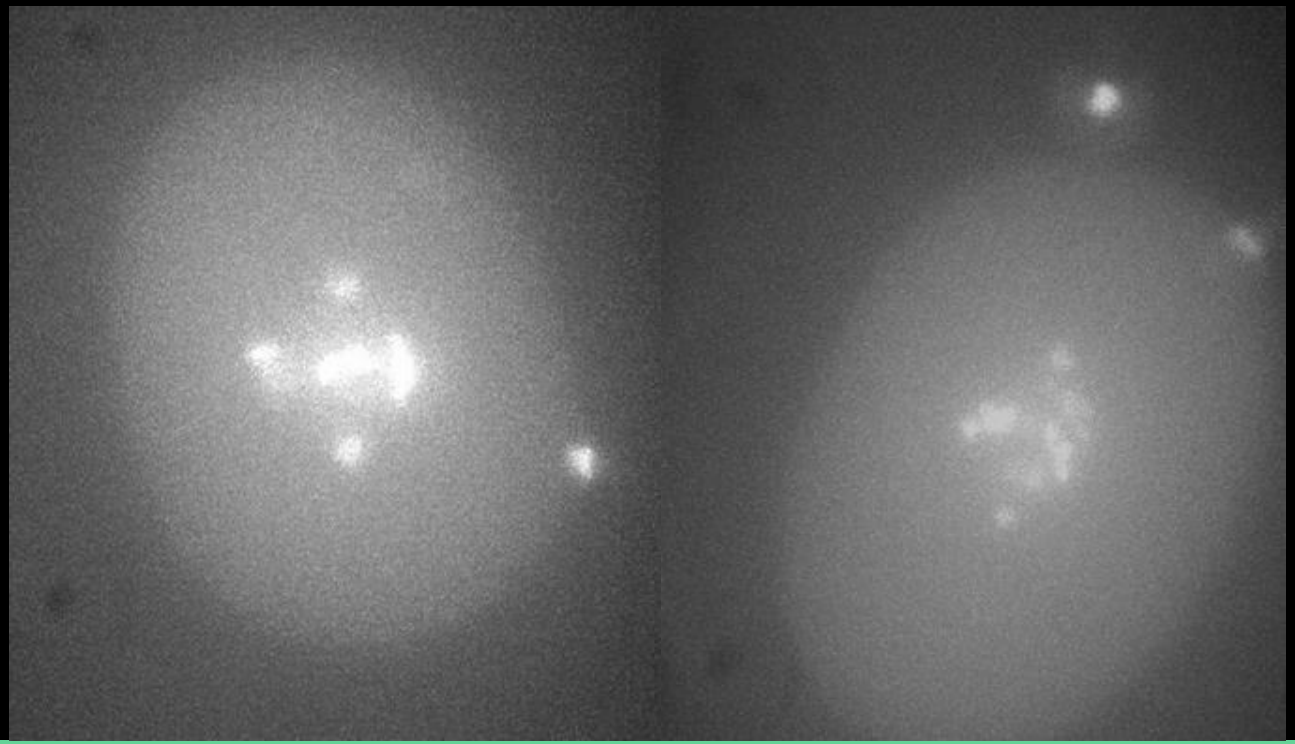
Western Blot of RNAi Through Feeding Protocol



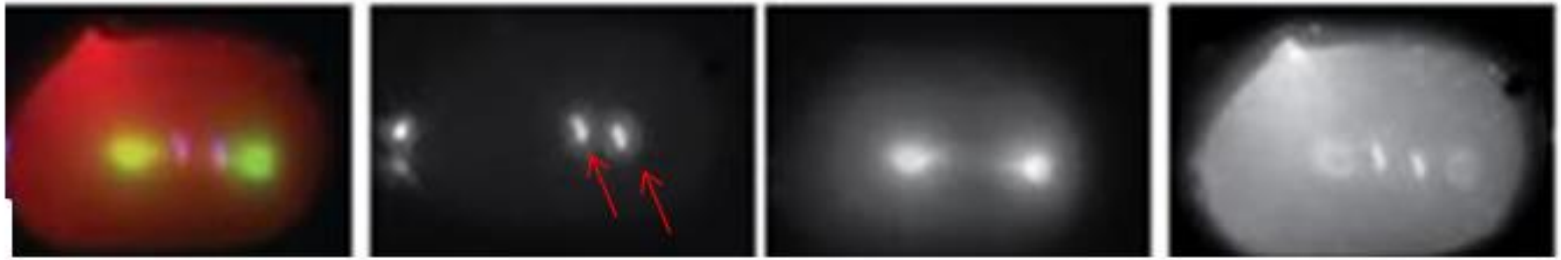
HCP-3 RNAi 1st Cellular Division

Wild Type

RNAi knockdown

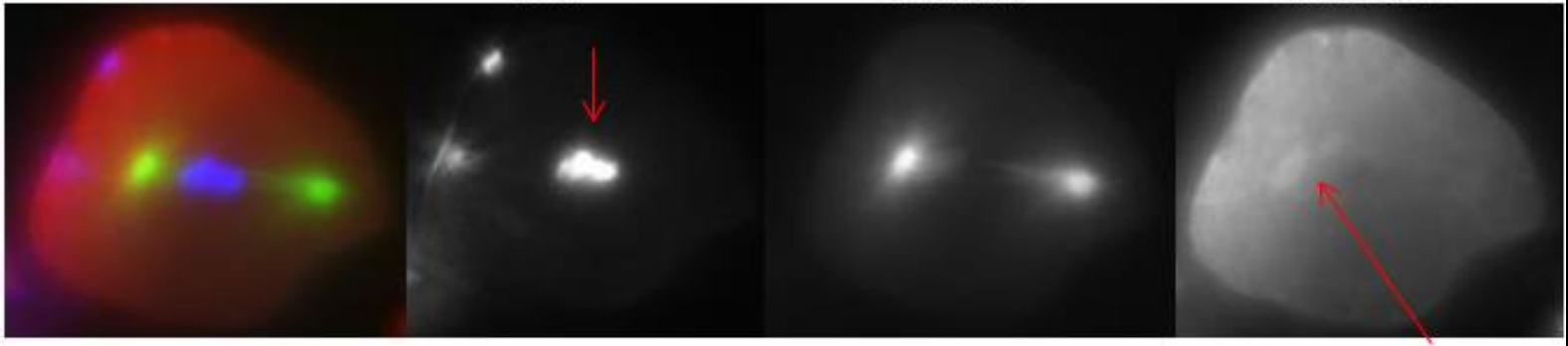


Anaphase in Wild Type



DNA a-Tubulin Anti-HCP-3

Anaphase in RNAi knockdown



Nonspecific binding of the antibody to the spindle

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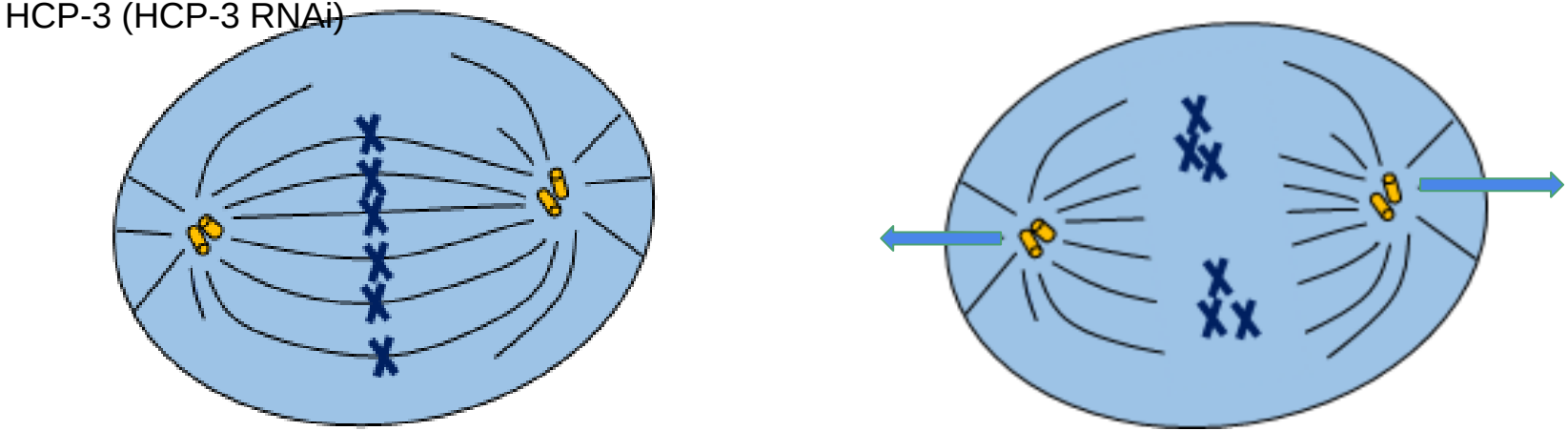
Concluding Remarks

- HCP-3 localizes to the centromere in mitosis
- Chromosome alignment and segregation is dependent on HCP-3
- Embryos lacking HCP-3 result in aneuploidy and embryonic lethality

With HCP-3 (WT)

Without HCP-3 (HCP-3 RNAi)



Future Directions

- Perform similar experiment for CPAR-1
  - Localization of CPAR-1 in the embryo using CPAR-1 specific antibodies
  - Functional live imaging of CPAR-1 depleted embryos
- Differential roles of HCP-3 and CPAR-1 will be examined to tease out additional roles “CENP-A” may be playing in the cell

Acknowledgements

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References

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Thank you!

Questions?