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

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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
Outline of Talk

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5. Immunofluorescence Assay

6. Live Imaging Assay

7. Conclusions and Future Directions
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**

[Image: 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
CENP-A

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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
Immunofluorescence Methodology

1. Make slides w/ embryos
   Dissect 30 worms on poly-K slides.

2. Freeze Crack embryos
   Add cover slip/place slides in liquid nitrogen/Flick off cover slip with razor blade.

3. Fix slides
   Place slides in -20 degree C MeOH fix.

4. Secondary Antibody
  Expose samples to secondary antibodies (anti:FITC/CY3.5, etc.)

5. Primary Antibody
   Expose samples to primary antibodies (anti:HCP-3/CPAR-1/Tubulin, etc.)

6. Wash with PBST

7. Wash with PBST

8. Wash with PBST

9. Wash with PBST

10. Hoechst
    Apply Hoechst to label DNA.

11. Mount Slides
    Apply photo-stable media/coverslip/seal w/ nail polish

12. Image
    Using Epi-Fluorescent Scope

13. Block
    Block with AbDil to prevent non-specific binding.
Western Blot of RNAi Through Feeding Protocol
HCP-3 RNAi 1st Cellular Division

Wild Type

RNAi knockdown
Anaphase in Wild Type

Anaphase in RNAi knockdown

DNA  a-Tubulin  Anti-HCP-3

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
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
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References


Thank you!

Questions?