Investigating the Role of Two CENP-A Homologs during Cell Division in the nematode *C. elegans*

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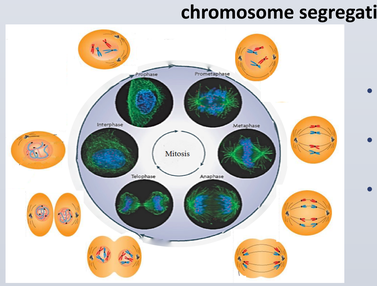
Abstract

CENP-A is a highly conserved Histone-H3 like protein, critical to centromere specificity and kinetochore assembly in all eukaryotes. Failure to properly produce or localize CENP-A leads to aneuploidy and cell death. In most organisms CENP-A has a single variant; however, in the nematode *C.elegans* CENP-A has two homologs, HCP-3 and CPAR-1. Based on previous studies, HCP-3 is responsible for specifying the centromere and thus critical for chromosome segregation in mitosis. CPAR-1's role however remains to be elucidated, albeit CPAR-1 is known to be essential as CPAR-1 mutants are embryonic lethal. The first step in understanding the role that CPAR-1 plays in embryonic development is to characterize where CPAR-1 localizes endogenously. To this effect, we are utilizing an immunofluorescence assay, which allows us to visualize chromosomes, microtubules, and the CENP-A homologs in the developing embryo to get a sense of where these proteins localize in the dividing cells. To date, we have imaged the cell cycle of endogenous embryos using immunofluorescent labeling of HCP-3, in conjunction with tubulin and DNA staining to label spindle microtubules and chromosomes respectively. HCP-3 depleted cells have also been imaged and compared to wild-type to assess the efficacy of RNAi depletion as indicated by HCP-3 signal levels and to assess the phenotypic consequence of HCP-3 depletion. In the near future we hope to repeat these types of experiments with newly generated CPAR-1 antibodies as well as CPAR-1 specific gene knock down reagents. Here, we will describe our current progress, future molecular strategies and experimental design leading up to our ultimate objective to assess the role of CPAR-1 in cell division. Through these studies, we will better understand what role CPAR-1 plays in embryonic development, and perhaps gain insight into a divergent role for CENP-A not yet characterized.

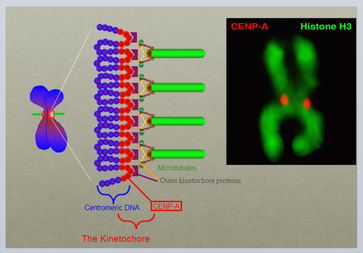
Background

CENP-A is a highly conserved Histone-like centromeric protein critical for proper chromosome segregation in eukaryotes.

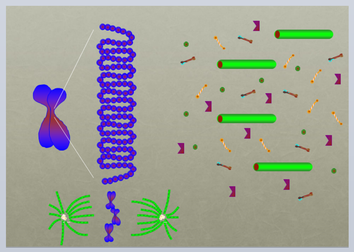


Mitotic Cell Division[1]

- Without CENP-A aneuploidy and cell death will occur.
- CENP-A is necessary for mitosis but not necessary for meiosis.
- Other roles for CENP-A are not known because knockouts and depletions are lethal.[2]



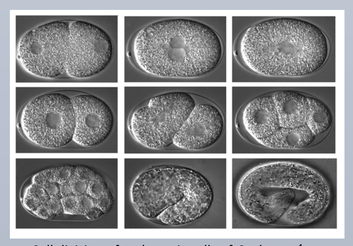
Chromosome with CENP-A[3]



CENP-A Depleted Chromosome[3]

***Caenorhabditis elegans (C. elegans)* provide an excellent model system to study cell division in general, and to tease out additional roles that CENP-A may play.**

- Large embryos
- Reach sexual maturity very quickly
- Very responsive to RNAi
- Two CENP-A homologues



Cell division of embryonic cells of *C. elegans*[4]

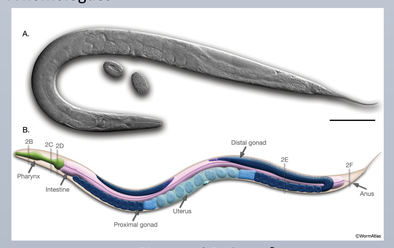
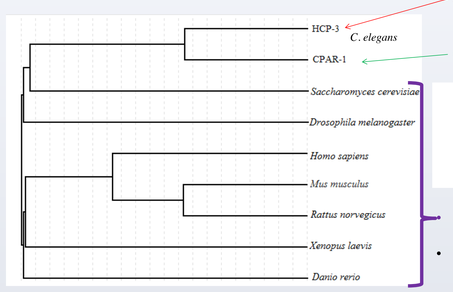


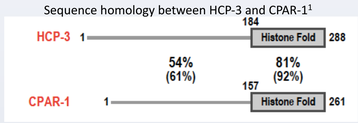
Diagram of *C. elegans*[5]

There are two CENP-A homologs in *C. elegans*



- HCP-3 plays the functional role of the centromeric protein in mitosis.
- The role of CPAR-1 is currently unknown.

Sequence homology between HCP-3 and CPAR-1[1]



- Most eukaryotes have just one CENP-A homolog.
- *C. elegans* provides a unique opportunity to characterize additional roles for CENP-A by studying the divergent homolog CPAR-1.

Are there divergent functional roles for HCP-3 and CPAR-1?

The Approach to test the Functional Roles of CENP-A Homologs

1. Determine where HCP-3/CPAR-1 express and where HCP-3/CPAR-1 localize
   - -Immunofluorescence using HCP-3 or CPAR-1 specific antibodies
   - -Development of transgenic worms expressing GFP tagged HCP-3/CPAR-1
2. Determine the functional consequence of embryos lacking CPAR-1
   - -Immunofluorescence using RNAi or mutant strains
   - -Live Imaging using RNAi or mutant strains

Immunofluorescence Methodology

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

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

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

↓ Wash with PBS

Block
Block with AbDil to prevent non-specific binding.

Wash with PBST →

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

Wash with PBST →

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

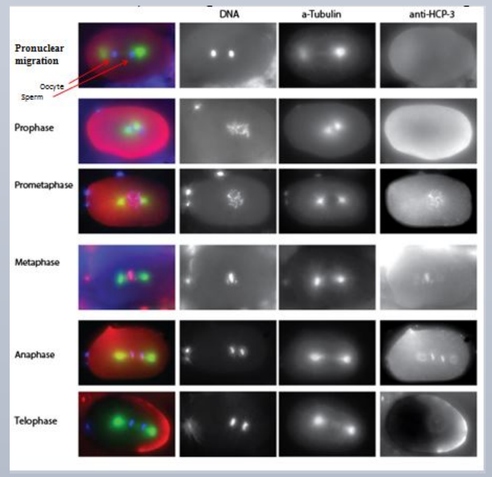
↓ Wash with PBST

Hoechst
Apply Hoechst to label DNA.

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

→ Image
Image Using Epi-Fluorescent Scope

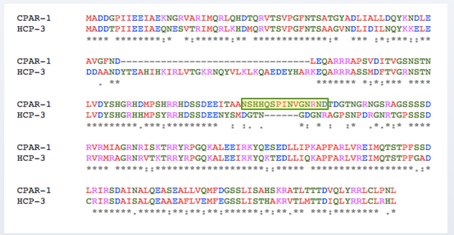
Wild-type embryos at various stages in the first mitotic division



HCP-3 localizes to centromeres during prometaphase → Anaphase in mitosis.

CPAR-1 specific antibodies still need to be tested using this approach.

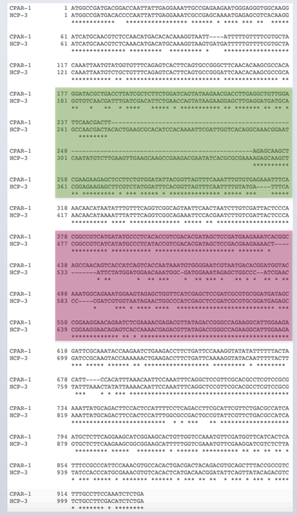
Developing a CPAR-1 Specific Antibody

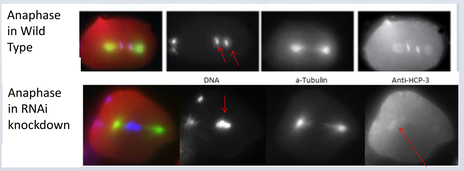


The highlighted region is the region we will be targeting to create the CPAR-1 specific peptide.

RNAi of CENP-A Homologs

Nucleotide sequence alignment to determine optimal dsRNA for HCP-3 and CPAR-1 specific RNAi targeting.





-The chromosomes in the wild type separate and start heading toward opposite poles. The chromosomes in RNAi knockdown remain clumped together.

-HCP-3 localizes at the centromeres in the wild type embryo whereas HCP-3 is absent in the RNAi knockdown cell.

Conclusion and Future Directions

C. elegans provide a unique model to study CENP-A

The divergent role of CPAR1 from HCP3 remains to be determined

HCP3 localizes to centromere from prometaphase through anaphase in mitosis

Preliminary data shows that HCP-3 is absent in the RNAi knockdown cell.

Assays have been optimized

HCP3 RNAi embryos fail to separate chromosomes during anaphase of mitosis

In the future we will:

Create transgenic worms for CPAR1 and HCP3

CPAR1 peptide has to be tested and optimized

Compare embryo images from both wild type and CPAR-1 and HCP3 RNAi knockdown to determine localization and functional role

Small regions of non-homology between the two CENPAs may provide a means to deplete them independent of one another and provide a way to detect them independent of one another with specific antibodies

References

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Acknowledgments

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