

Live Imaging and Analysis of Wild-Type Versus HCP-3 Depleted Embryos in the Nematode, *Caenorhabditis elegans*

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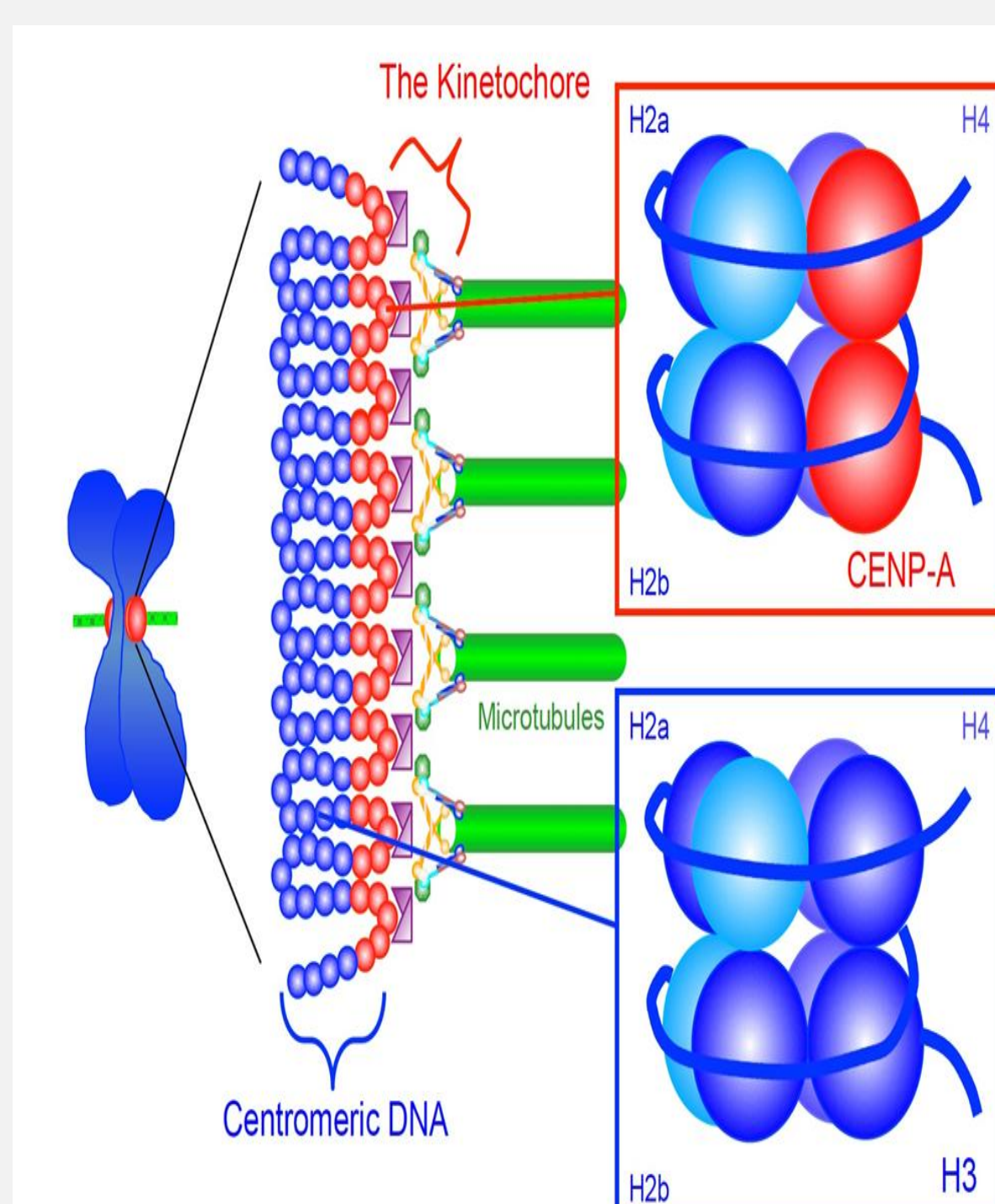
Background

CENP-A and Mitosis

Spindle microtubules connect to Histone H3 variant CENP-A in mitosis

C. Elegans has 2 homologs of CENP-A

- HCP-3 – critical for mitotic chromosome segregation
- CPAR-1 – role has yet to be determined

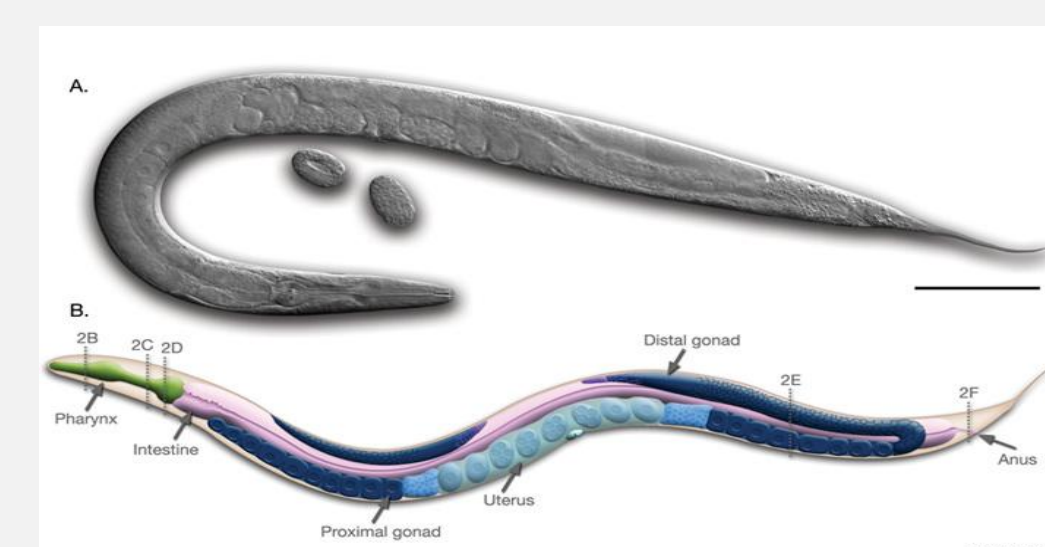


Hypothesis:

There are diverging functional roles for HCP-3 and CPAR-1.

Objective:

To characterize the functional roles of HCP-3 and CPAR-1 in early embryonic.



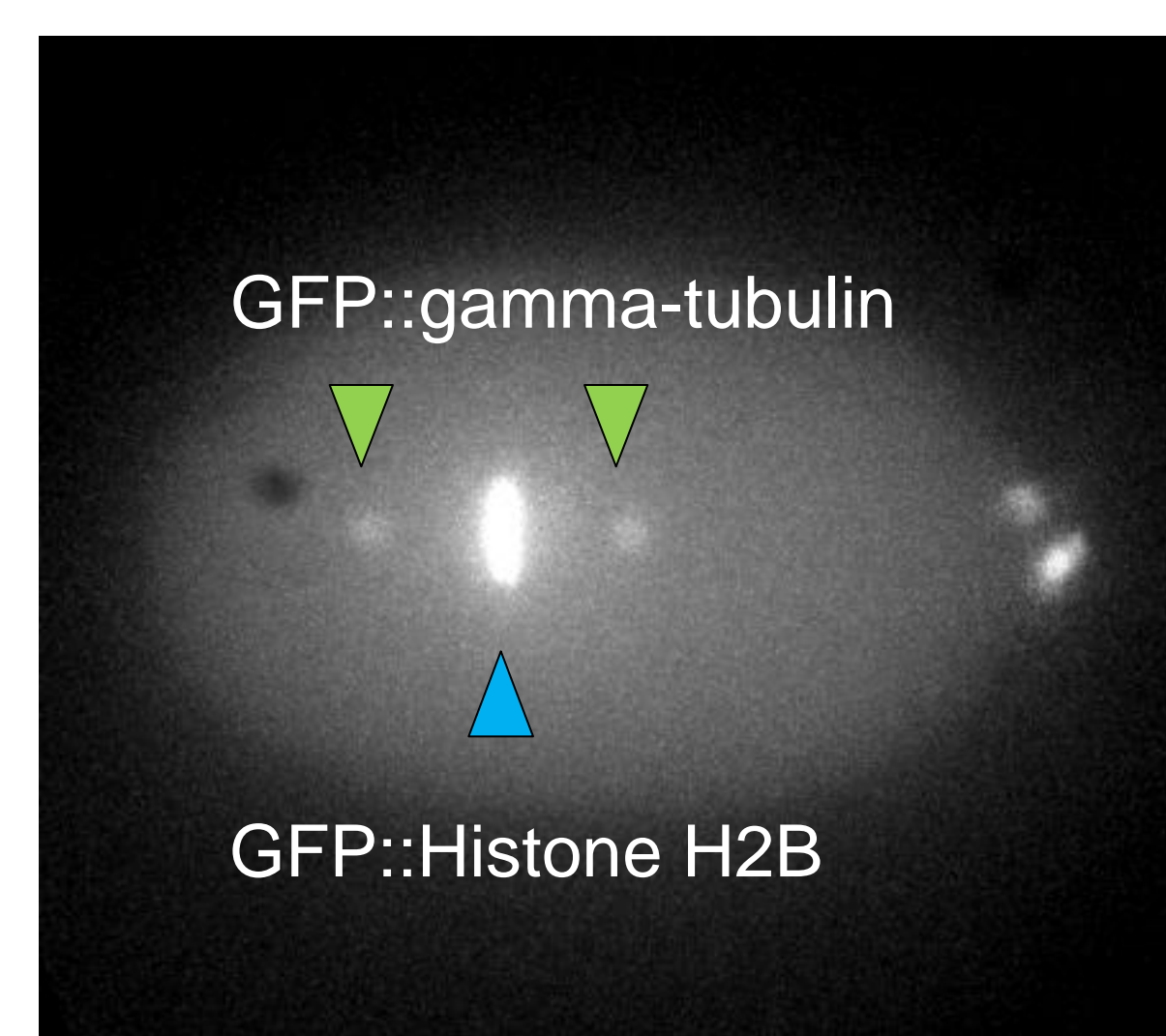
Methods

Caenorhabditis elegans : 2 Types Imaged

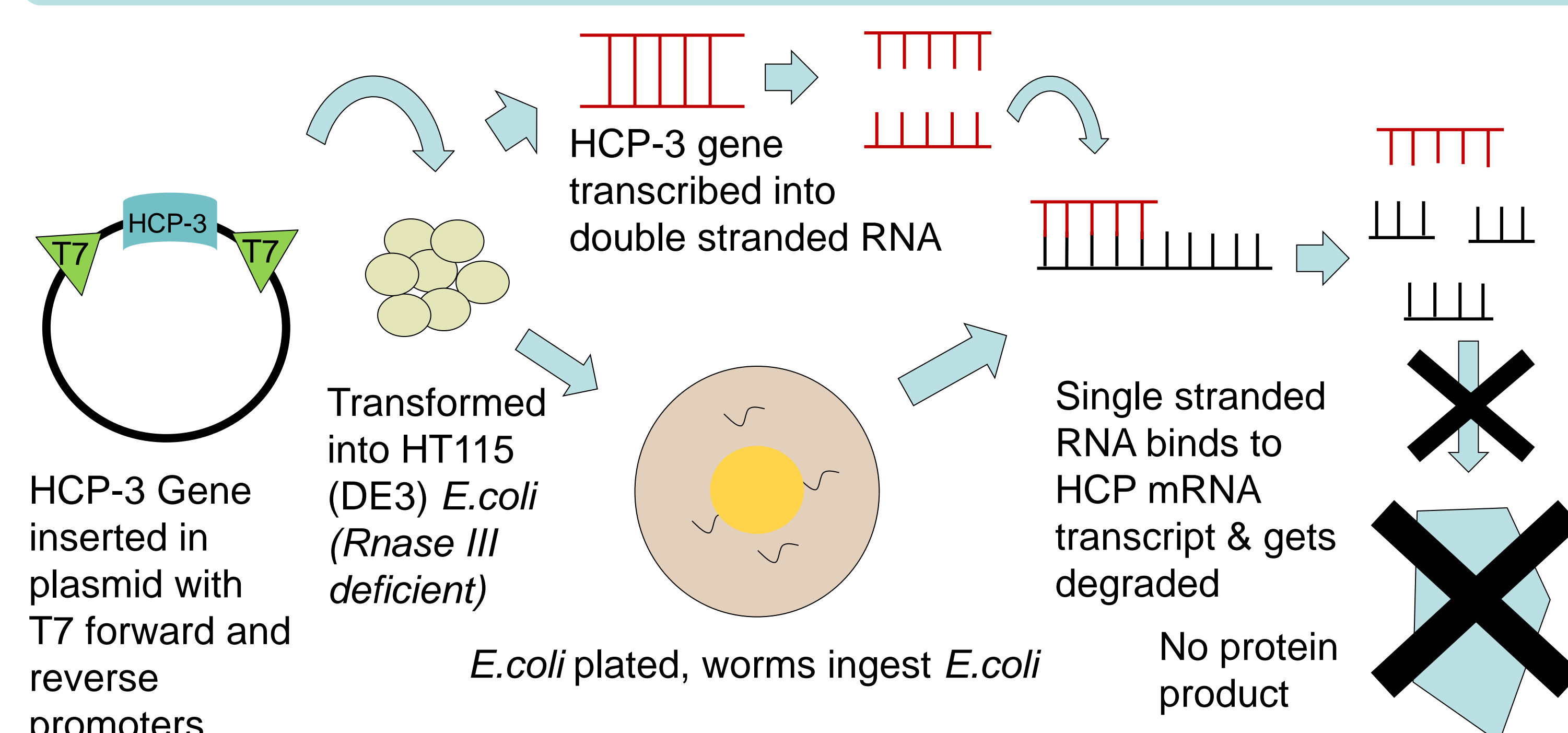
• GFP::Histone H2B/GFP::gamma-tubulin expressing wild-type (TH32)

• Allows visualization of chromosomes and spindle fibers

• HCP-3-depleted TH32



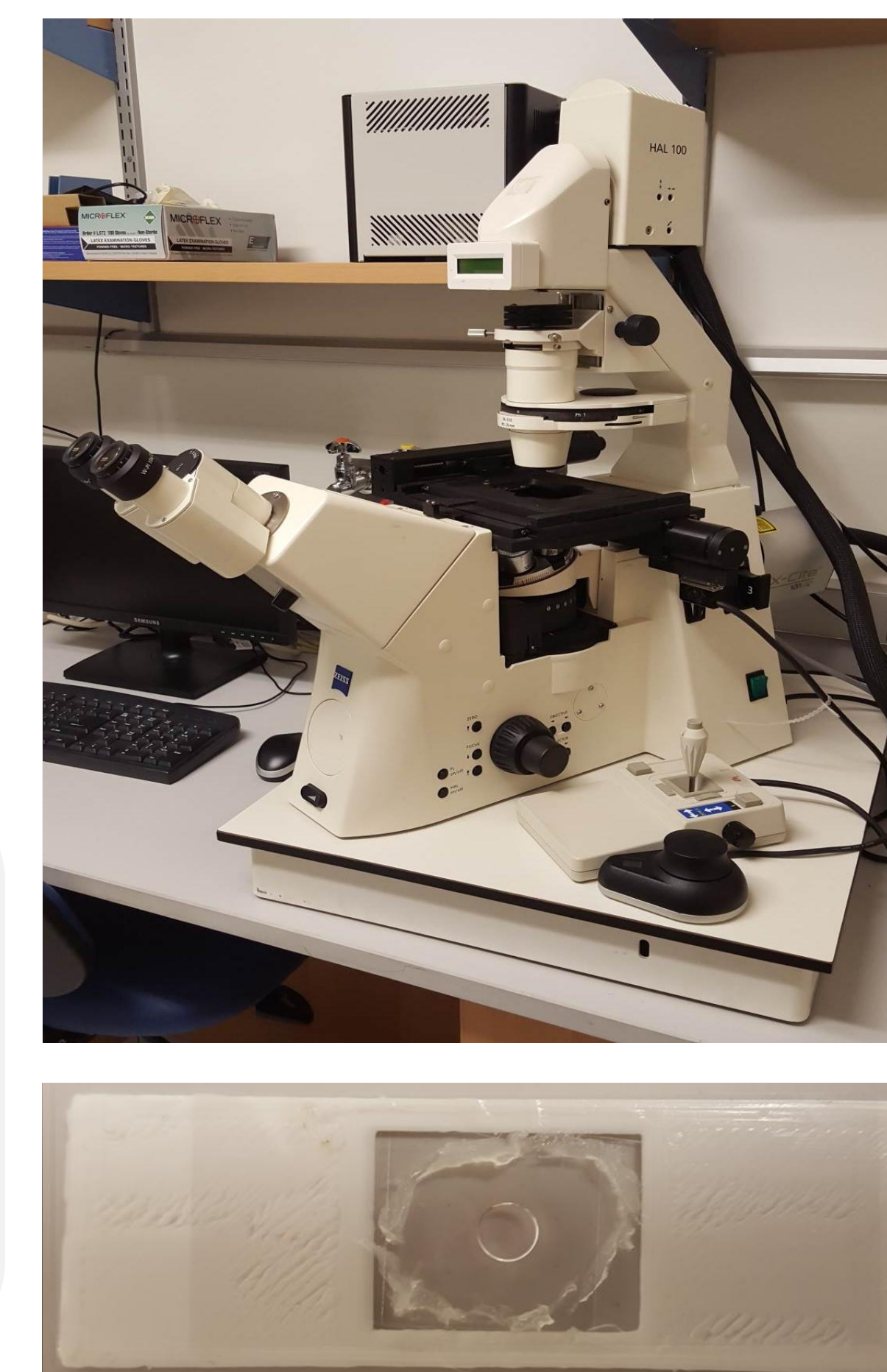
RNAi in *C. elegans*: Feeding Protocol



Live Imaging and Analysis

Zeiss Axiovert 200M epi-fluorescent microscope

- 30 second intervals
- FITC filter (to excite GFP)



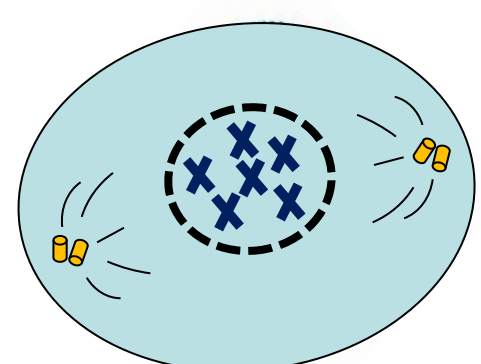

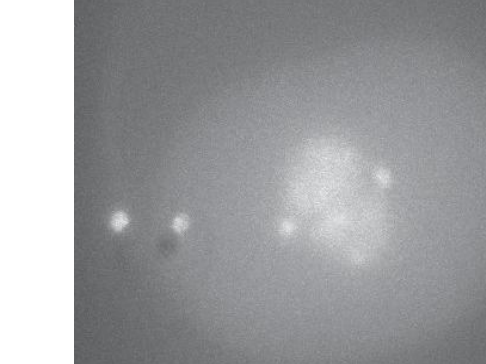


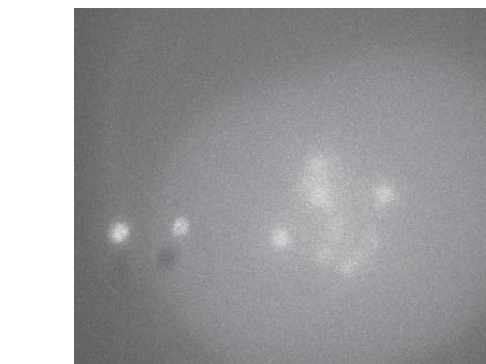
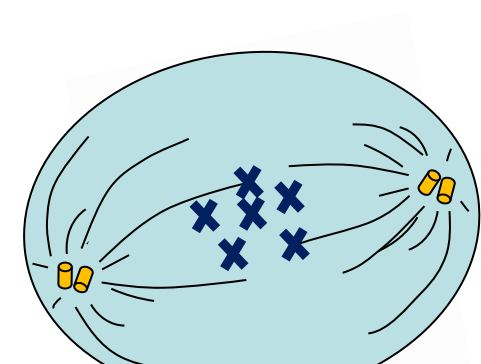
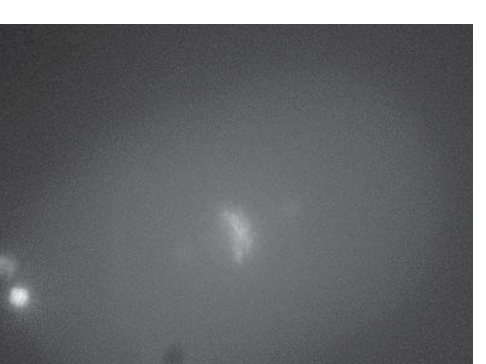
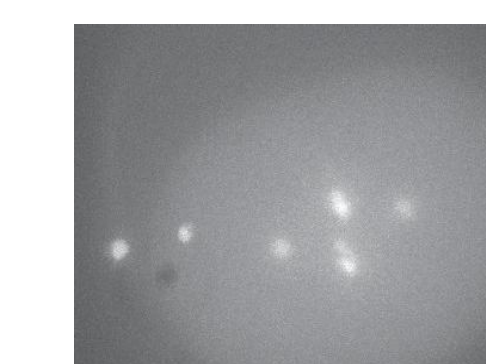
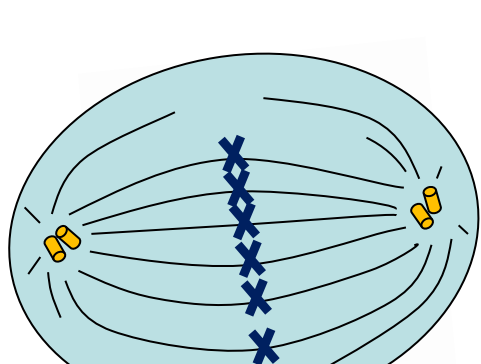

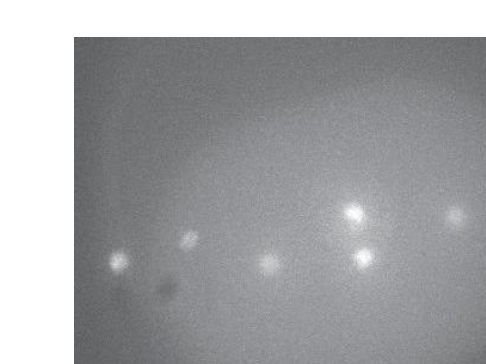



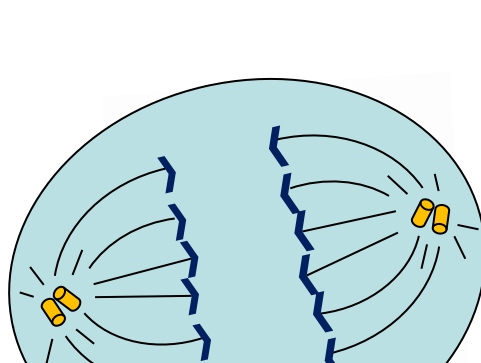
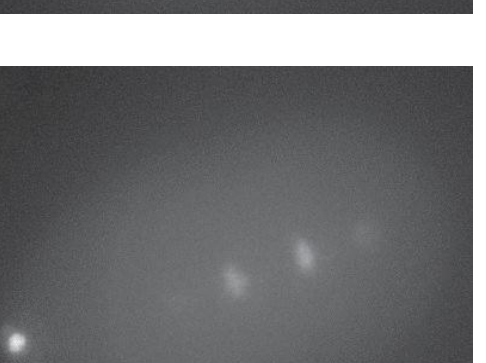
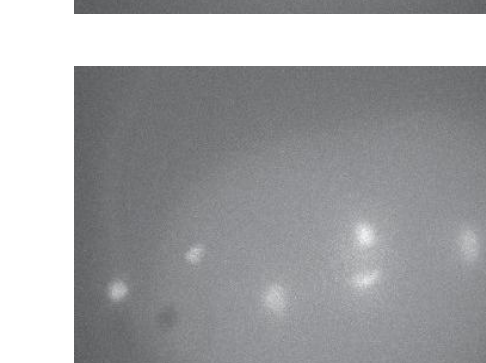
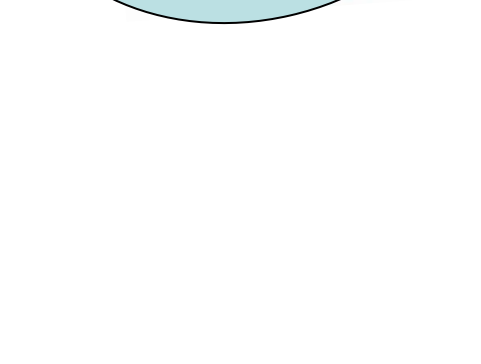

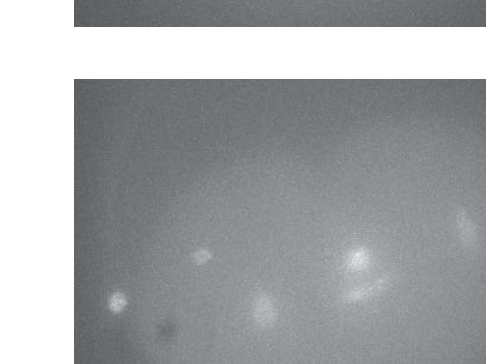
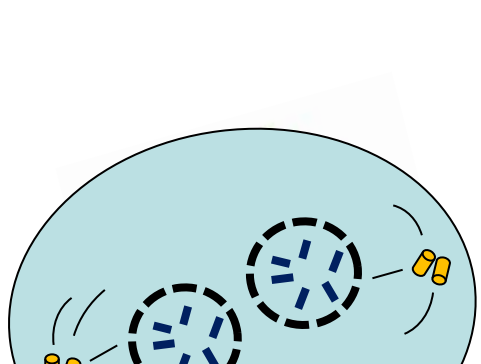
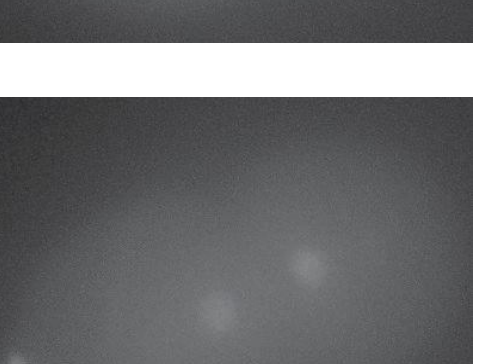
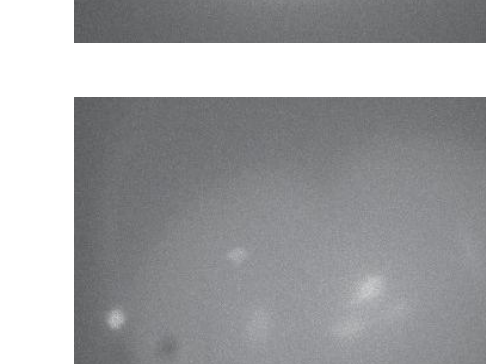
2 Types Imaged:

WT TH32 vs. HCP-3 Depleted Embryos

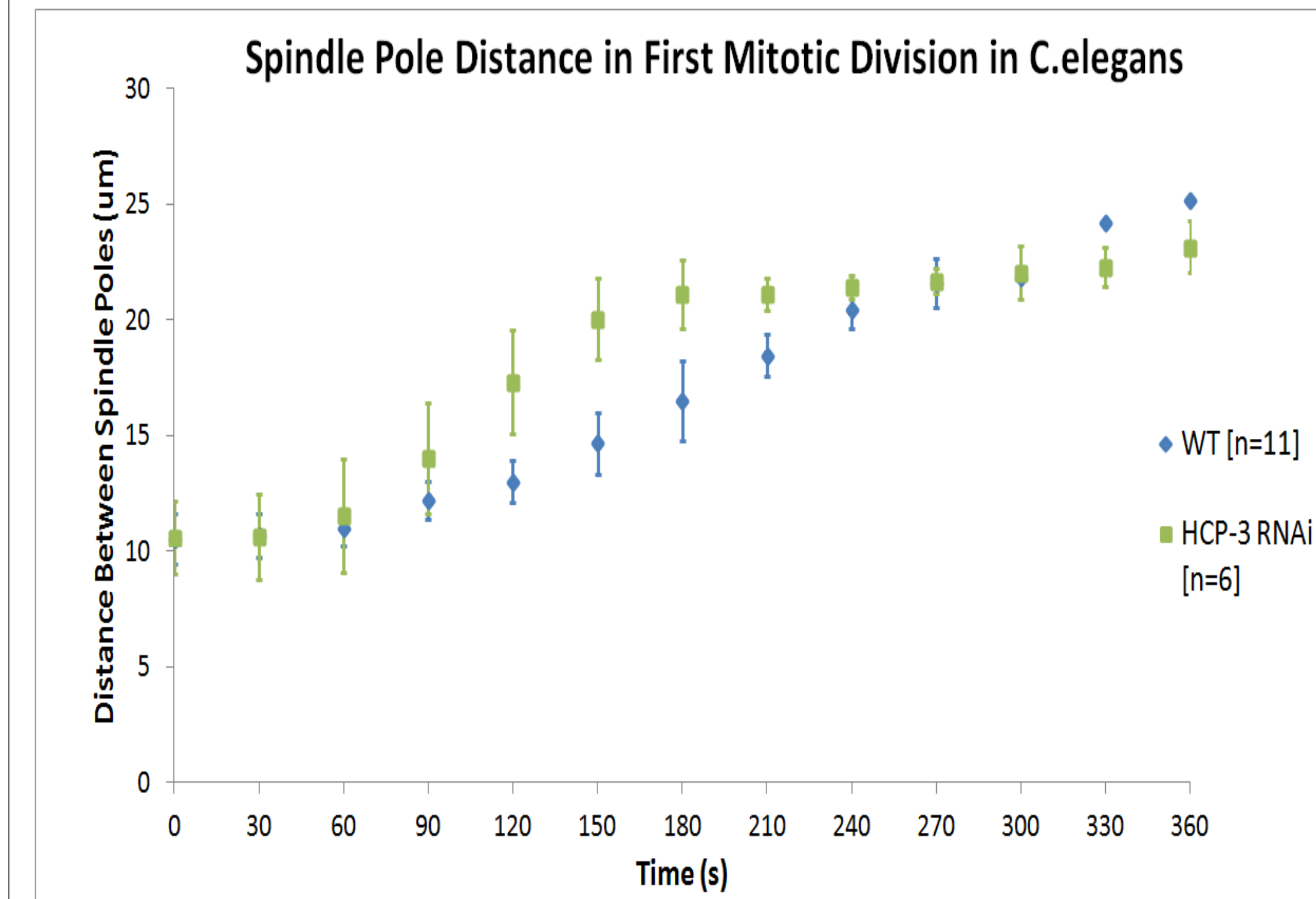
Analysis of spindle pole movement via ImageJ

Results

Chromosome Segregation in WT vs HCP-3 Depleted Embryos

	WT	HCP-3 Depleted	Time (minutes)	HCP-3 Depleted Phenotypes
			0:00	• Onset of nuclear membrane breakdown
			0:30	
			1:30	
			2:30	• Chromosomes fail to align in the center of the cell
			3:30	• Microtubules fail to connect to kinetochore
			4:30	• Spindle poles shoot out from chromosomes
			5:30	
			6:30	• Cytokinesis forces genetic material into either cell

Spindle Pole Separation



- Spindle poles migrate gradually toward poles in WT embryos.
- At t=120s, spindle poles in HCP-3 RNAi shoot out toward the poles.

Conclusions

- Chromosomes fail to separate properly, in HCP-3 depleted embryos resulting in uneven separation of genetic material and therefore embryonic lethality.
- In WT embryos, spindle poles gradually move poleward during mitosis whereas spindle poles in HCP-3 depleted embryos shoot out poleward prematurely likely due to the lack of attachments of spindle microtubules to the kinetochore of chromosomes.

Further studies include:

- Characterizing functional role of CPAR-1 in early embryonic development by assessing CPAR-1 depleted embryos
- Assessing level of depletion in RNAi feeding protocol using a Western blot

References

Monen J, Hatersley N, Muroyama A, Stevens D, Oegema K, et al. (2015) Separate Cleaves the N-Tail of the CENP-A Related Protein CPAR-1 at the Meiosis I Metaphase-Anaphase Transition in *C.elegans*. PLoS ONE 10(4).