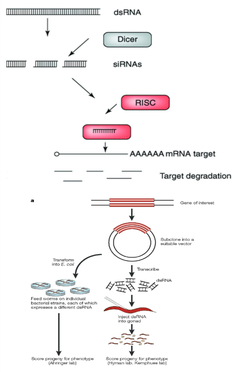
Comparing the efficacy of RNAi injection vs. feeding in the nematode *C. elegans*

JJ Fritsch, Matt Von Bargen, Samantha Davis, Julianna Herman Advisor: Dr. J. Monen
School of Theoretical and Applied Science, Mahwah, NJ, 07430

RNA-mediated interference (RNAi) is a process by which RNA molecules inhibit gene expression via specific degradation of mRNA transcripts. Since its discovery nearly 20 years ago, researchers have utilized this understanding to specifically knock-down genes of interest. In the nematode *C. elegans,* several RNAi techniques have been developed, including injection of double stranded RNA (dsRNA) and feeding of bacteria expressing dsRNA, yet a systematic study determining the efficacy of the two approaches has not been conducted to date. In this study, we test the efficacy of the injection and feeding method to knock-down an essential mitotic protein HCP-3, and assess the effectiveness of this strategy for use in future studies. Knock-down effectiveness will be measured by western blot analysis and immunofluorescence, and the phenotypic consequences will be assayed by embryonic lethality and live-imaging of cell division in mCherry:Histone-H2B & GFP:a-tubulin transgenic worms. Currently, HCP-3 RNAi feeding demonstrates that knock-downs result in 100% embryonic lethality and western blot analysis reveals that HCP-3 has been depleted to ~5% that of wild-type levels. We are currently in the process of testing the injection method and replicating the feeding results.

RNA-mediated interference (RNAi)

•inhibit gene expression by causing the destruction of specific mRNA molecules
•when mRNA molecules are no longer present, protein cannot be made



Mechanism of RNA-mediated Interference (RNAi)

- dsRNA recognized and chopped into siRNA pieces by the enzyme Dicer
- siRNAs bind to the RISC complex and find complementary mRNA
- RISC cleaves the mRNA transcript preventing translation & thus gene expression of the target transcript.

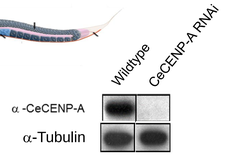
2 RNAi Strategies in *C. elegans*

The gene of interest is inserted into a vector with 2 flanking Bacterial Promoters (T7).
1) transformed into *E. coli*
-fed to the worms
2) transcribed into dsRNA
-injected into the worms

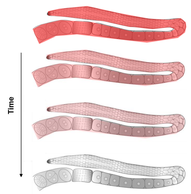
C. elegans as a model for studying protein depletion

-Easy to maintain
-Hermaphrodites
-self-fertilize
-progeny are identical to parent
-High levels of gene knock-down via RNAi

RNAi in *C. elegans* can deplete targeted proteins by >99%

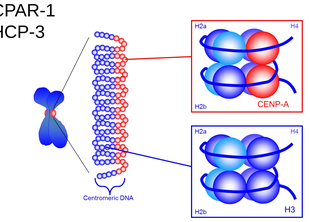


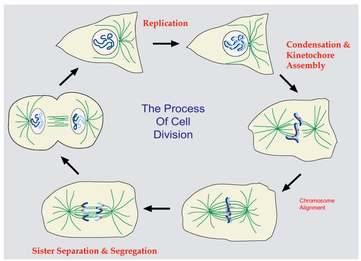
As the CeCENP-A is depleted by the RNAi, the amount of protein in the cell decreases until there is nothing left



CENP-A's role in mitosis

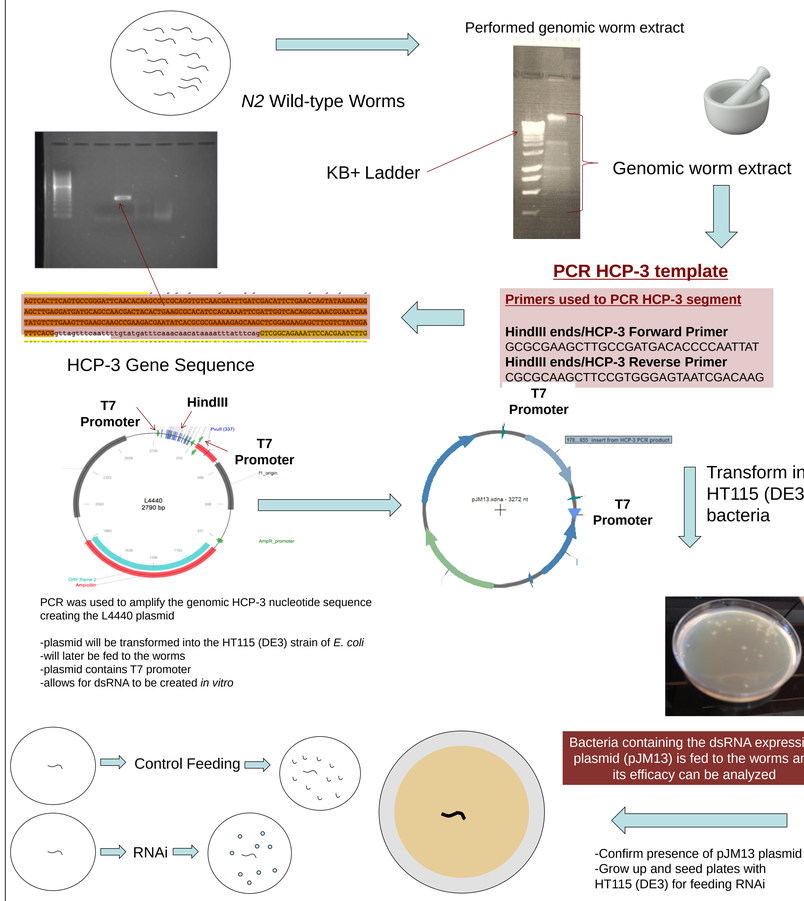
-CENP-A is a protein located within the centromere of a chromosome & essential for chromosome segregation.
-In *C. elegans,* two homologs of CENP-A exist:
-CPAR-1
-HCP-3





The Setup: Dual Pronged approach to creating and prepping dsRNA for RNAi feeding & injection comparison

RNAi Feeding – Bacteria containing the RNAi plasmid is fed to the worms and when digested will incorporate into the DNA.



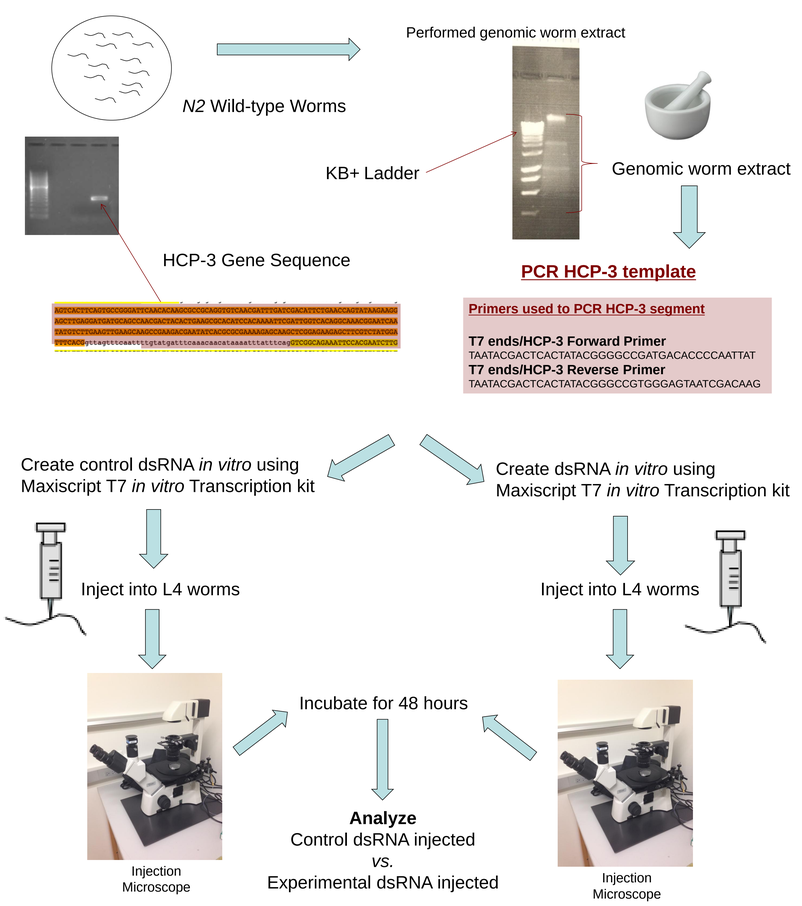
PCR was used to amplify the genomic HCP-3 nucleotide sequence creating the L4440 plasmid

-plasmid will be transformed into the HT115 (DE3) strain of *E. coli*
-will later be fed to the worms
-plasmid contains T7 promoter
-allows for dsRNA to be created *in vitro*

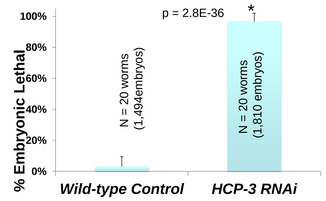
Bacteria containing the dsRNA expression plasmid (pJM13) is fed to the worms and its efficacy can be analyzed

-Confirm presence of pJM13 plasmid
-Grow up and seed plates with HT115 (DE3) for feeding RNAi

RNAi Injection – The RNAi plasmid will be injected directly into the worm gonad causing it to incorporate directly into the DNA



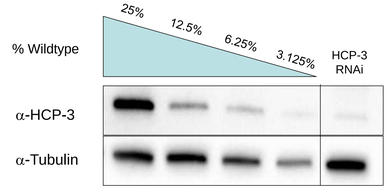
Embryonic Lethality Assay using Feeding Approach



$$Embryonic\ Lethality = \frac{\#\ of\ inviable\ embryos}{\#\ inviable\ and\ live\ worms}$$

Western Blot

1) 100 adult worms from RNAi feeding plate, 100 from control feeding plate to separate M9 tubes
2) Wash 3 times, spin, and remove supernatant
3) Remove liquid to 50uL and add 50uL 2xSB
4) Both tubes were sonicated for 20 minutes
5) Serial dilutions for control sample → 22uL of 1xSB in 5 tubes and 22uL from previous tube making each tube half as dilute as the prior tube
6) Ran SDS-Page



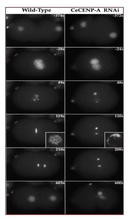
Depleted HCP-3 by ~97% using feeding protocol

Future Directions

Live Imaging

-Wild Type goes through the steps of Mitosis naturally and without any issues creating viable offspring

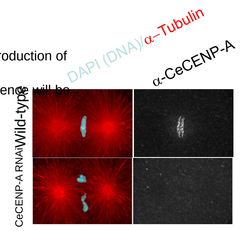
-Cell subjected to CeCENP-A RNAi through RNAi feeding or injection cannot complete Mitosis and dies



Immunofluorescence

-RNAi will stop the production of protein

-no fluorescence will be seen



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Acknowledgements

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