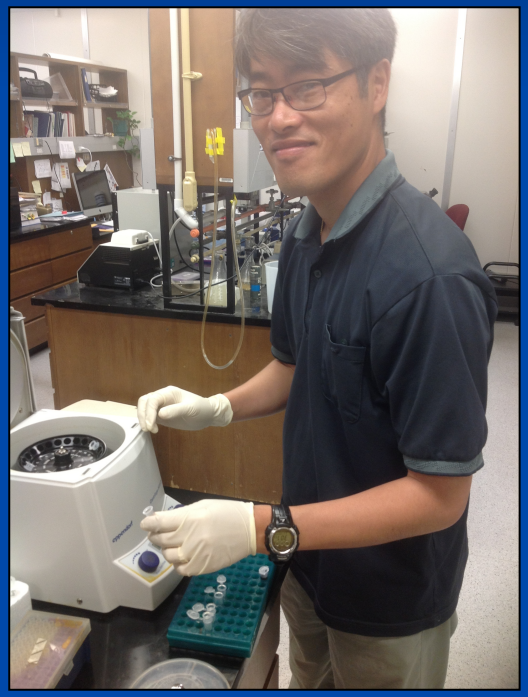
RET Summer 2013

RET Participant: Brendan Carroll

Location: Dr. Joel Rothman's Lab
UCSB Department of Molecular Cellular and Developmental Biology



Mentor:

Dr. Pan Young Jeong

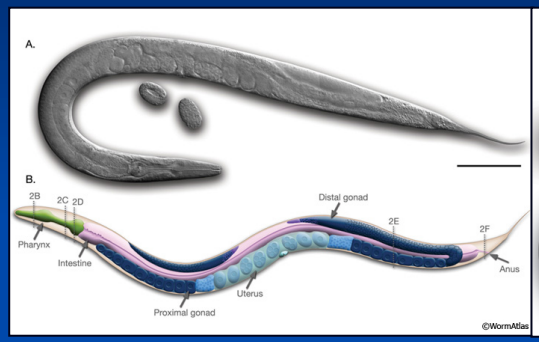
C. elegans

Dr. Joel Rothman

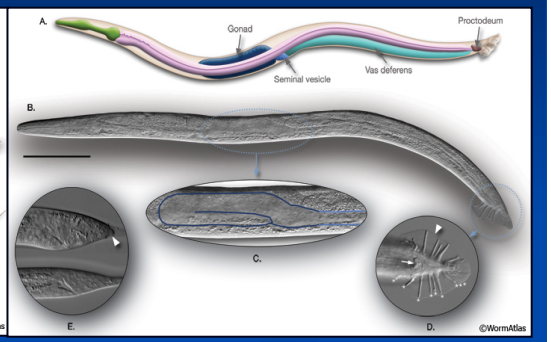
C. elegans: A Model Organism for Research

Ideal subject for genetics research;

- Life span 2-3 weeks
- Adults 1mm
- Transparent
- RNAi (introduced via inoculated bacteria)
- Genome completely mapped
- Hermaphroditic



hermaphrodite

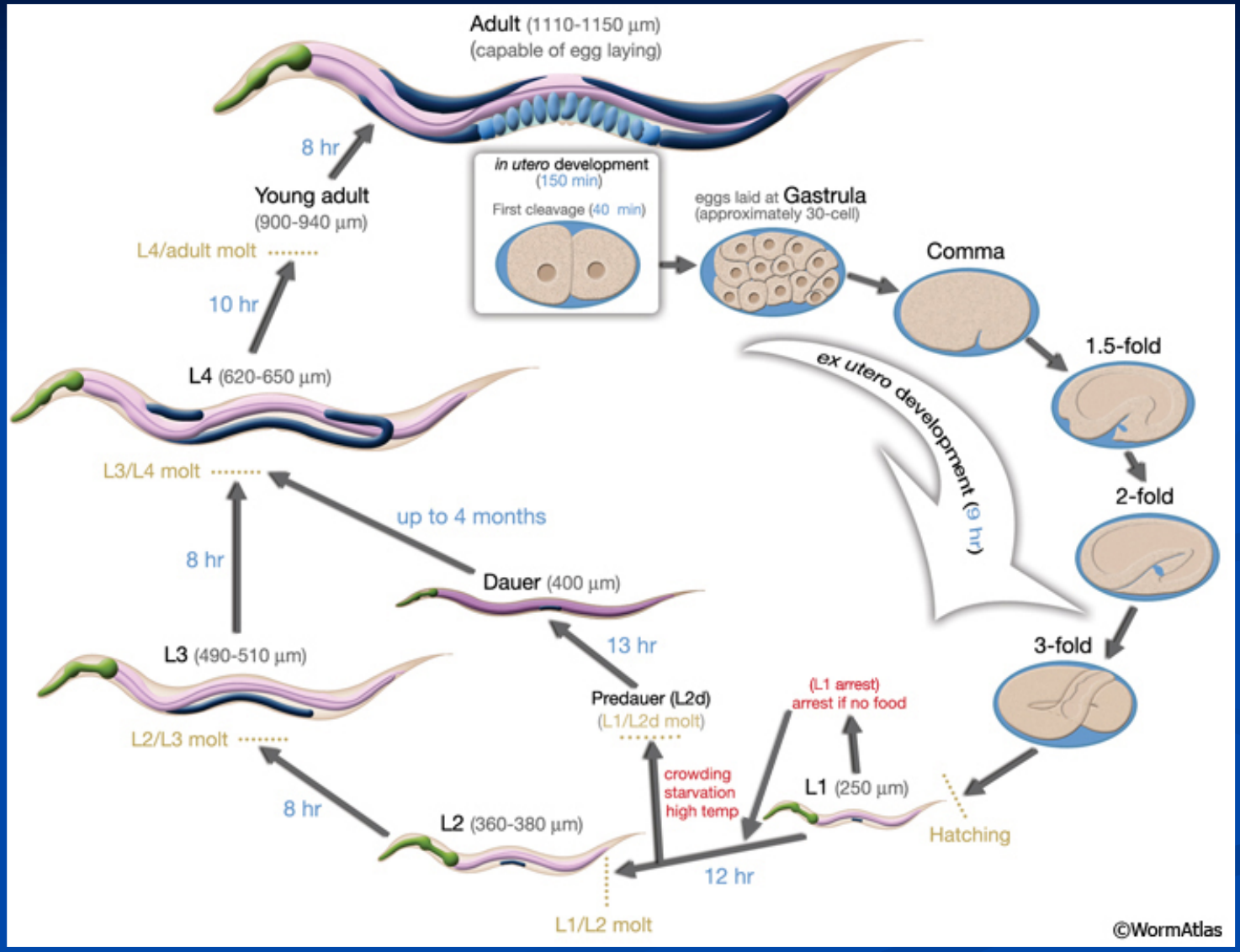


male

INTERESTING FACTS:

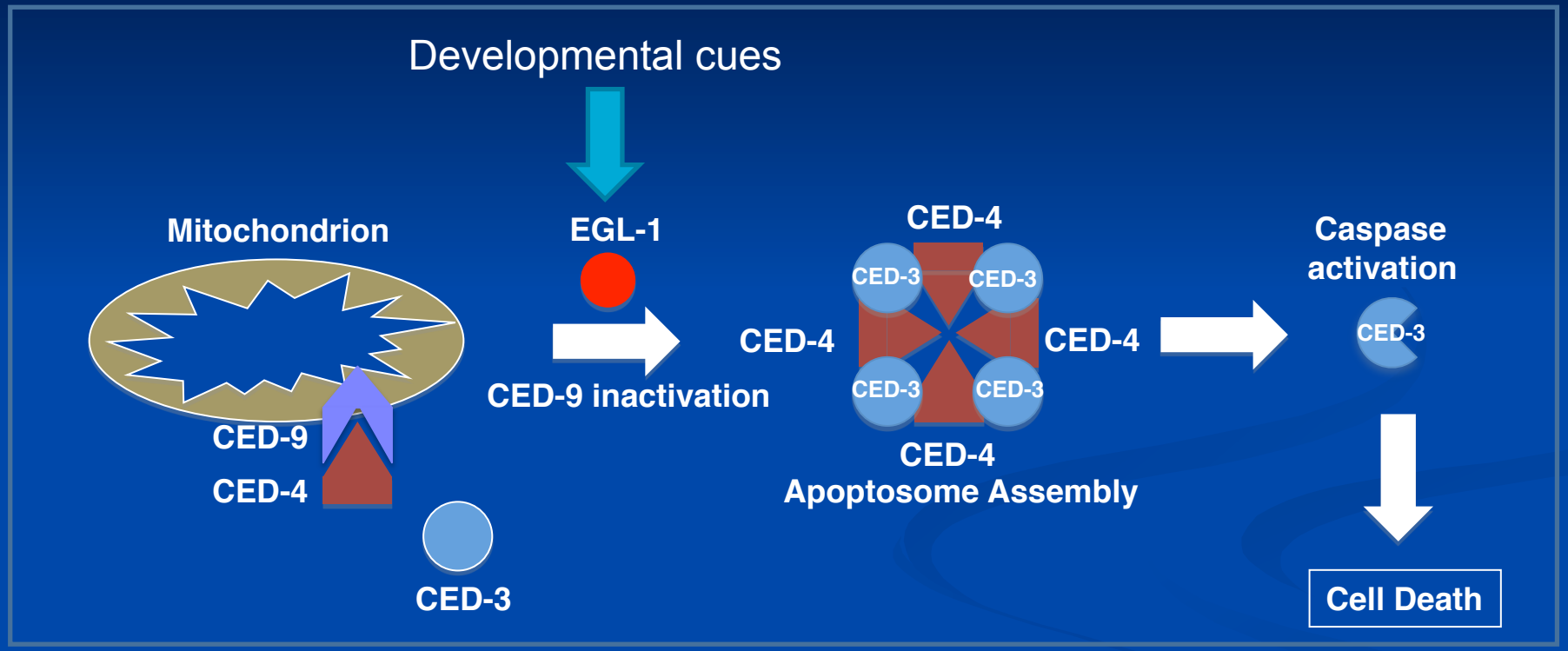
- Survives -80° C for 10 years
- Survived 2003 space shuttle Challenger disaster
- Descendants of the Challenger survivors traveled to space on the Endeavour in 2011

Life Cycle of *C. elegans*



PCD model in *C. elegans*

Pathway to Apoptosis (programmed cell death) discovered in *C elegans*



- Cancer
- Genetic birth disorders
- Parkinson's disease

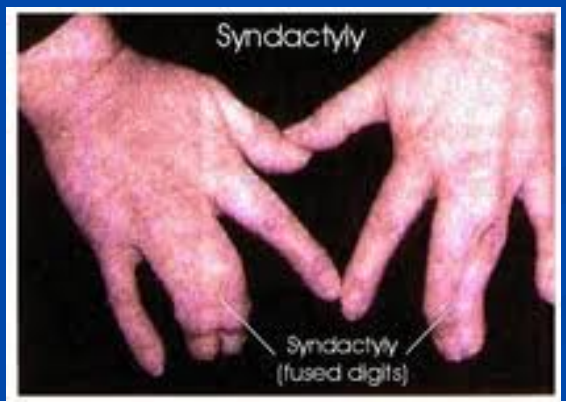
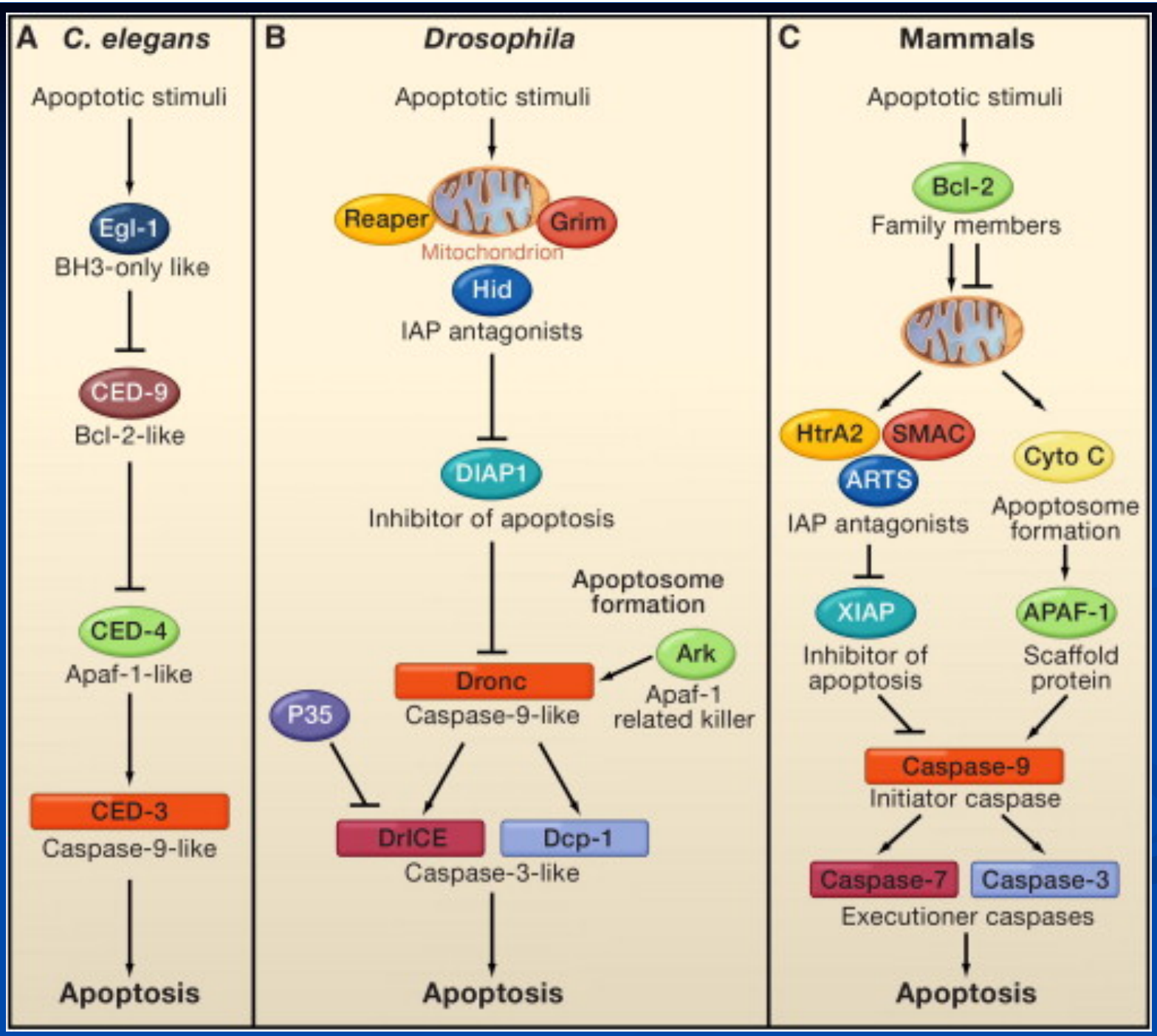
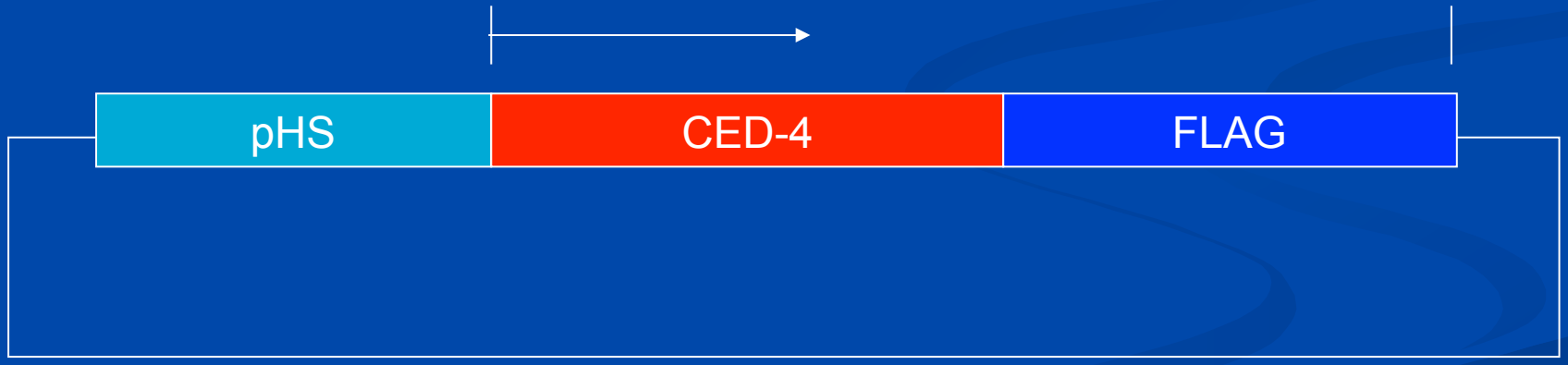


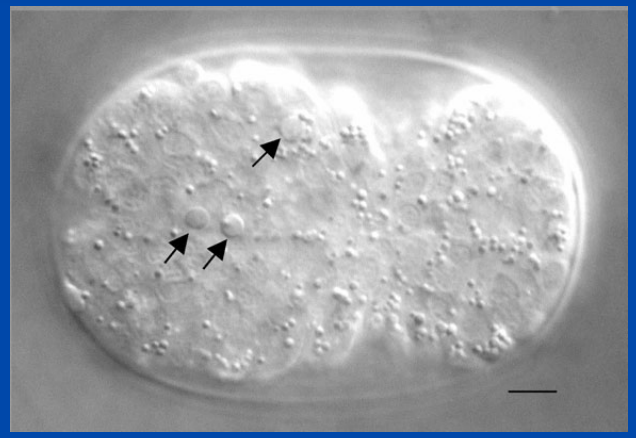
Diagram by Dr.Pan Young Jeong


A C. elegans
Apoptotic stimuli
Egl-1
BH3-only like
CED-9
Bcl-2-like
CED-4
Apaf-1-like
CED-3
Caspase-9-like
Apoptosis
B Drosophila
Apoptotic stimuli
Reaper
Grim
Mitochondrion
Hid
IAP antagonists
DIAP1
Inhibitor of apoptosis
Apoptosome formation
Ark
Apaf-1 related killer
Dronc
Caspase-9-like
P35
DrICE
Dcp-1
Caspase-3-like
Apoptosis
C Mammals
Apoptotic stimuli
Bcl-2
Family members
HtrA2
SMAC
ARTS
IAP antagonists
Cyto C
Apoptosome formation
XIAP
Inhibitor of apoptosis
APAF-1
Scaffold protein
Caspase-9
Initiator caspase
Caspase-7
Caspase-3
Executioner caspases
Apoptosis

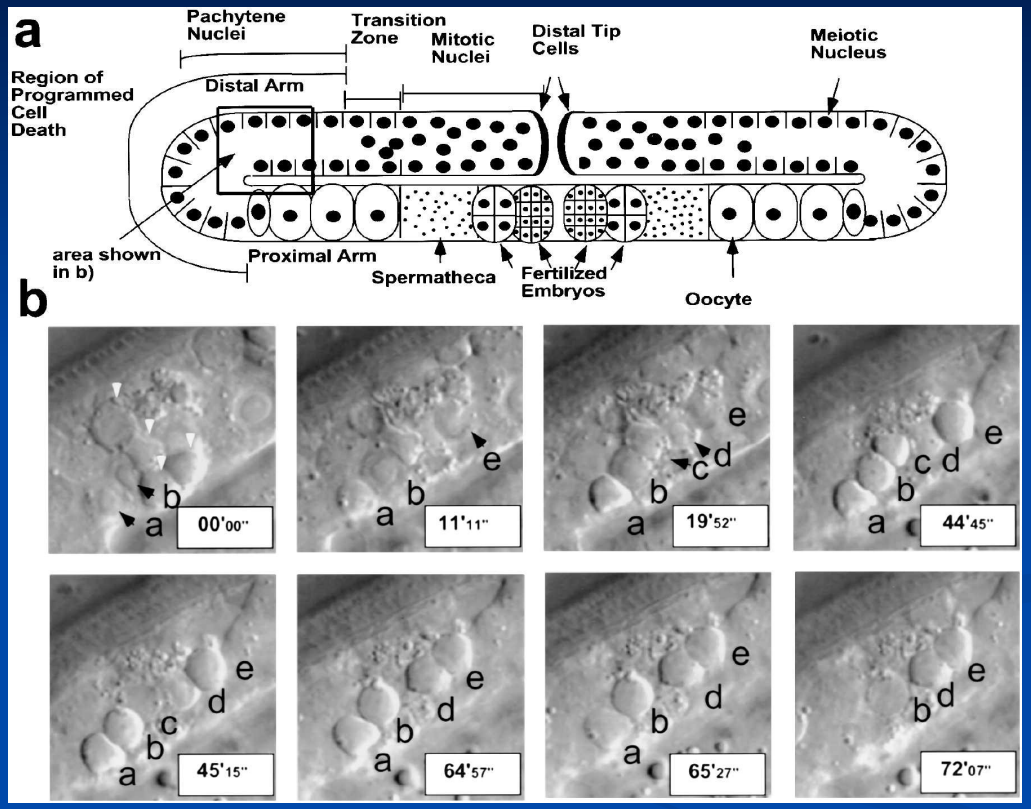
- This summer Dr.Pan-Young Jeong will have me help determine which conditions are the optimal heat shock and recovery times for identification of new CED-4 binding proteins, based on the identification of apoptotic cell corpses.
- ced-4(-);RNAi(some genes) mutant fertile phenotype will be compared with N2;RNAi(some genes) sterile phenotype
- The pHS;CED-4::FLAG is regulated by Heat-shock promoter (pHS).



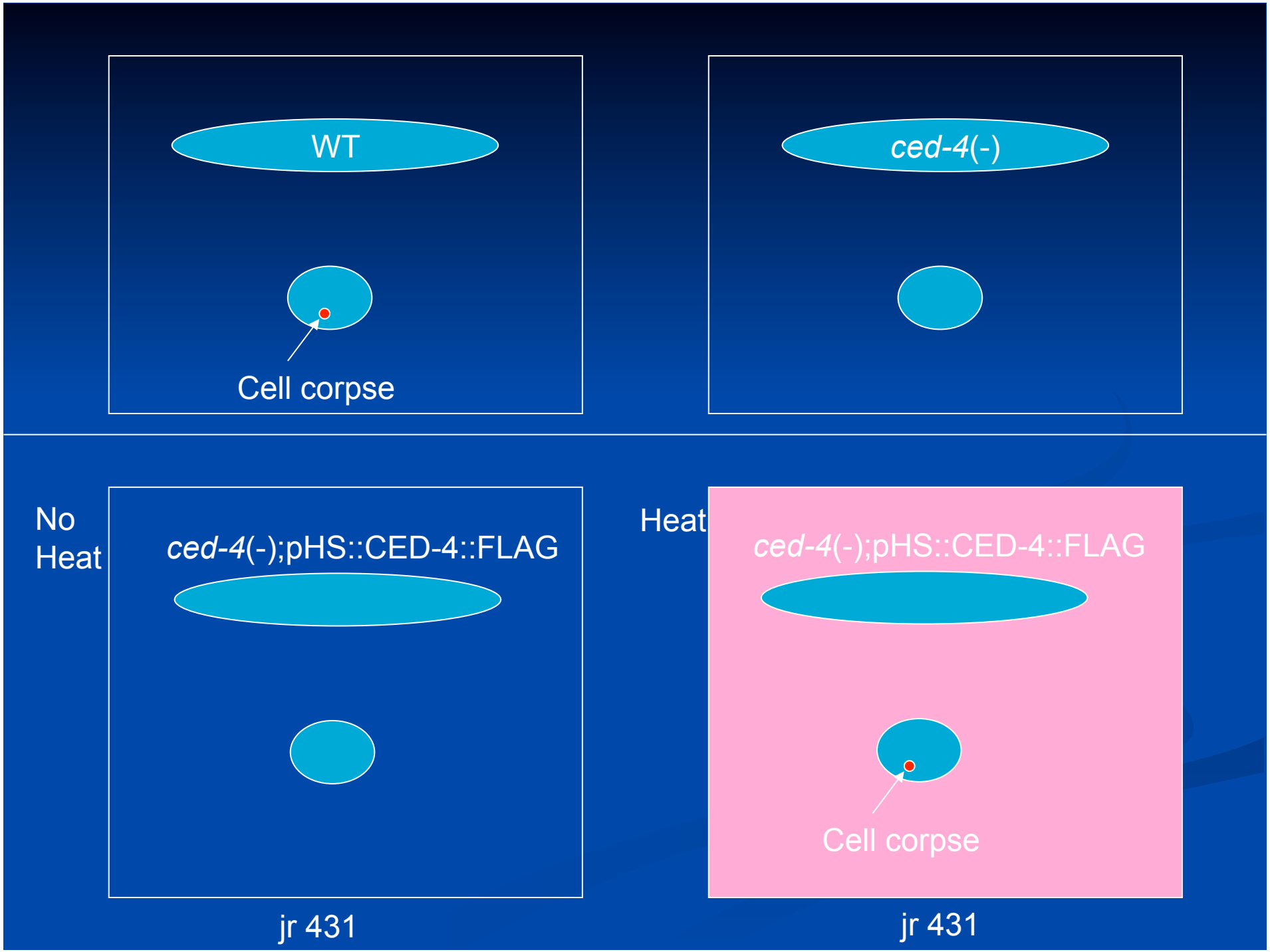
- We can use anti-FLAG base on FLAG to help identify and coimmuno precipitate the CED-4 binding proteins (We don't have anti-CED-4).



Somatic cell



Germ cell


WT
Cell corpse
ced-4(-)
No Heat
ced-4(-);pHS::CED-4::FLAG
jr 431
Heat
ced-4(-);pHS::CED-4::FLAG
Cell corpse
jr 431

Methods: Phase 1

1. Transfer (“pick”) jr431 AD worms to two plates (20worms/plate)

2. Place plates in 30º C incubator for dependent time (5, 10, 20, 30, 60 mins)

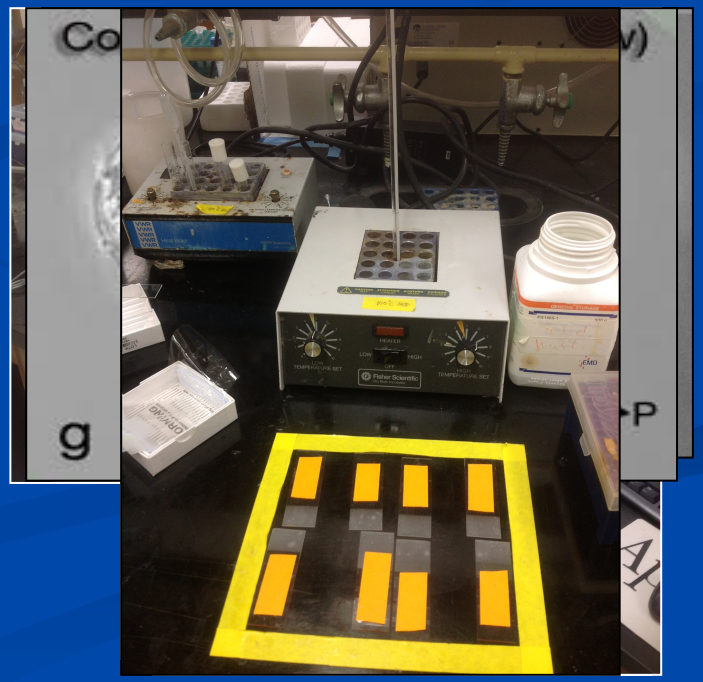
3. Place one plate in 20º C incubator

4. Transfer worm plate from 30º C incubator and allow a “recovery” time of 2 hours in 20º C incubator

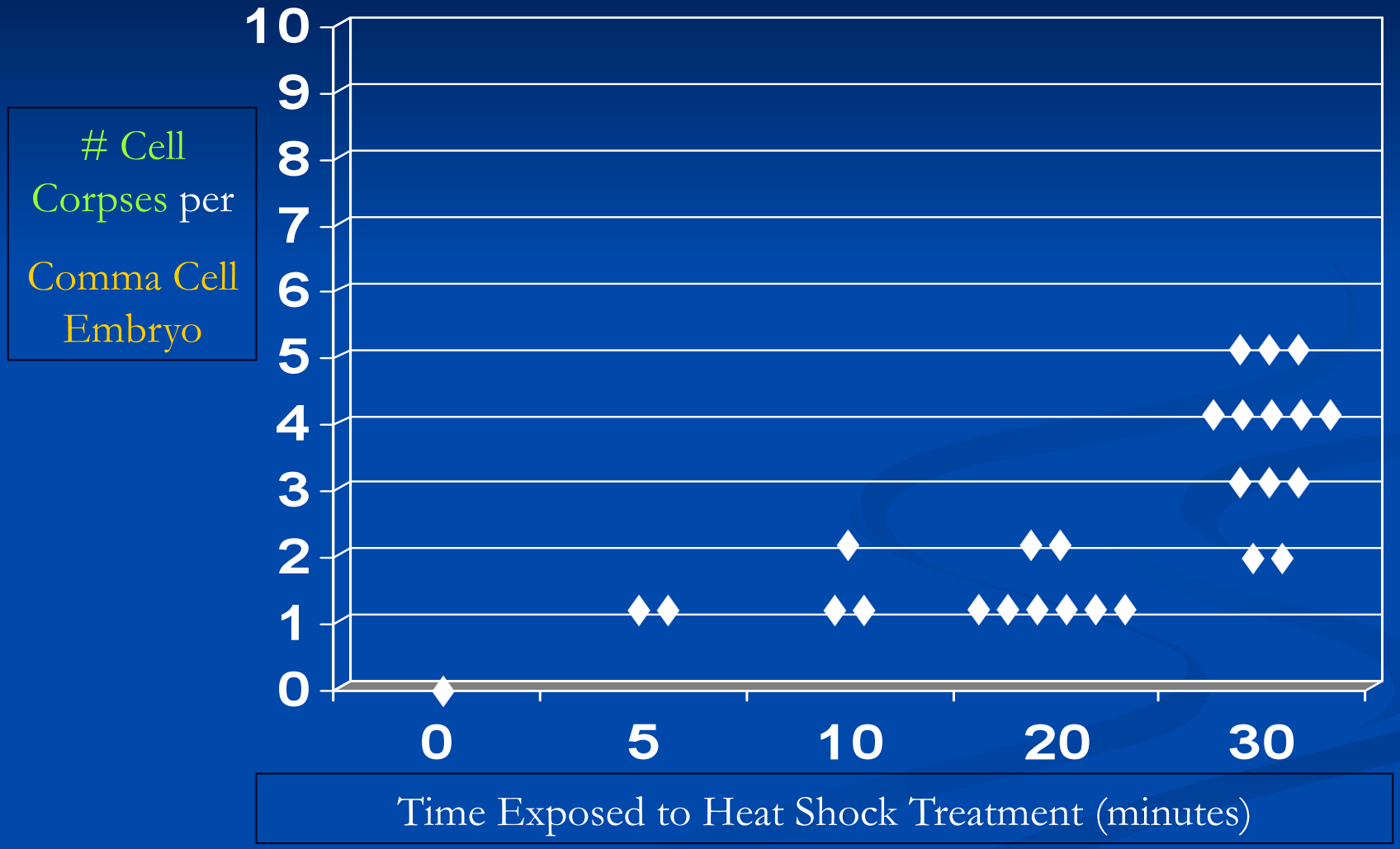
5. Prepare agarose slide for embryo viewing

6. Pick comma stage embryo from each plate

7. Observe and count the number of cell corpses on Zeiss high-resolution microscope (Differential Interference Contrast mode).



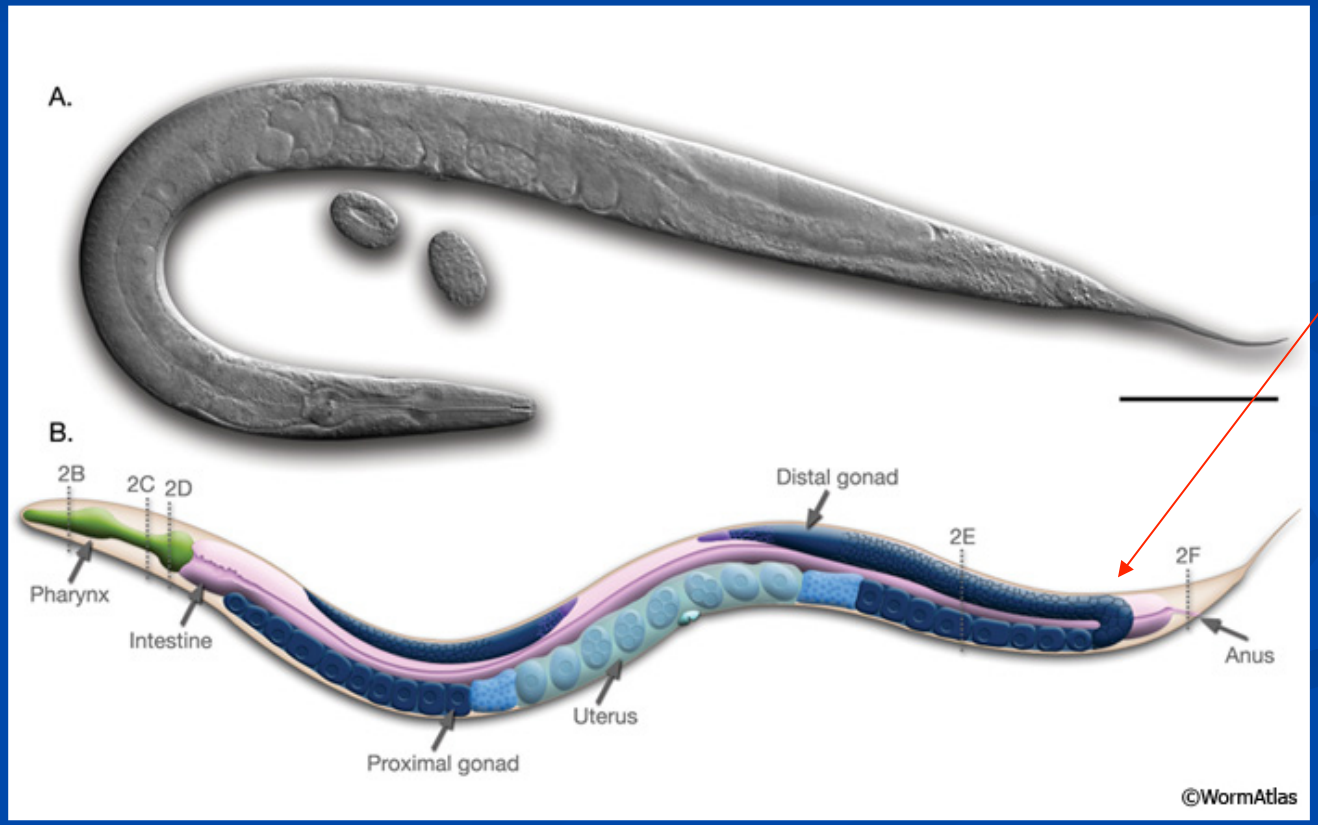
Cell Corpse Observations Post-Heat Shock Treatment



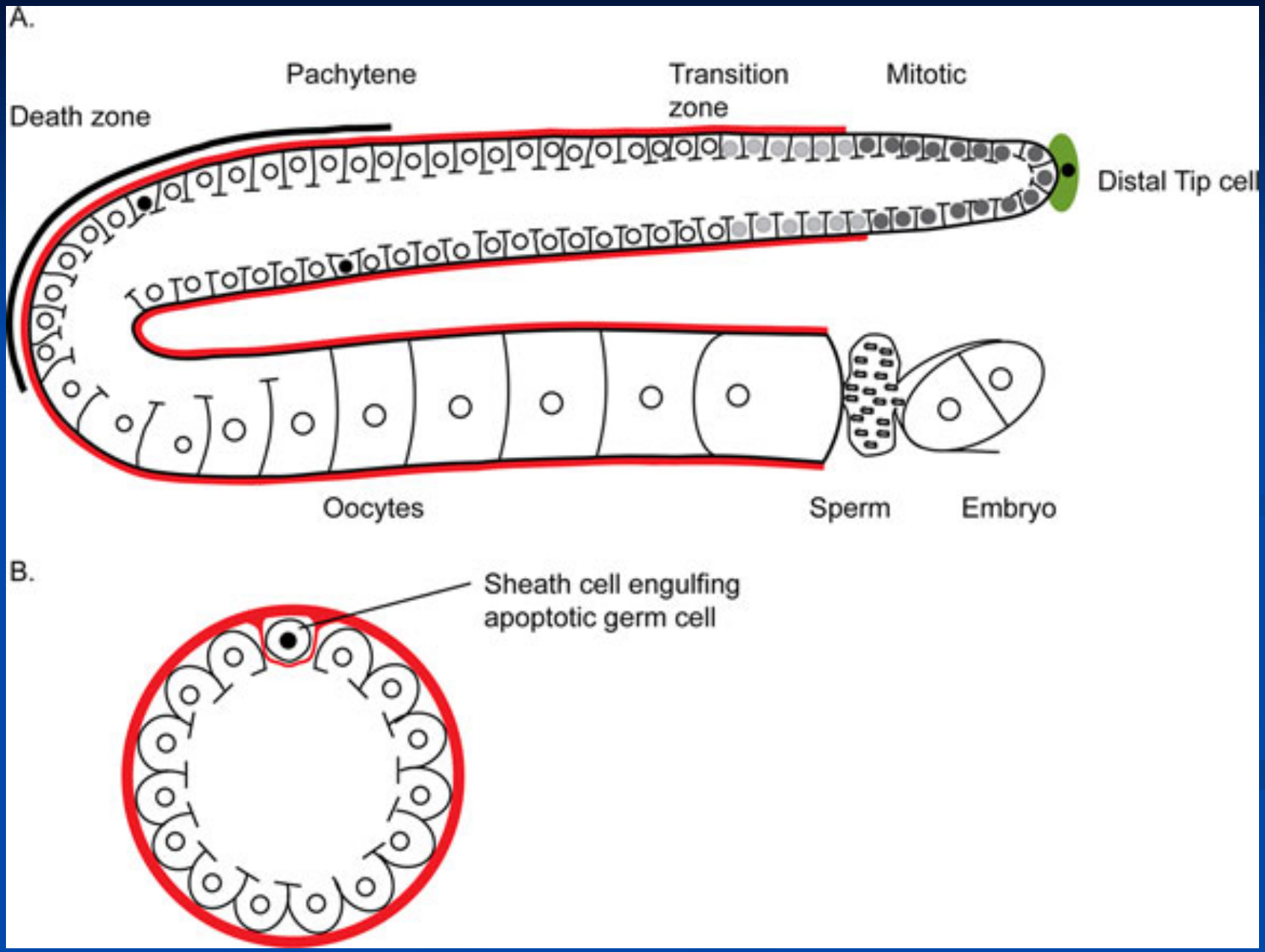
Total Embryos Observed: (10) (8) (9) (16) (13)

Next Step

- L4 stage ced-4(-);RNAi(some genes) worms treated to heat shock
- Examine "Death Zone" in gonad for apoptosis
- Fluorescent cell corpse

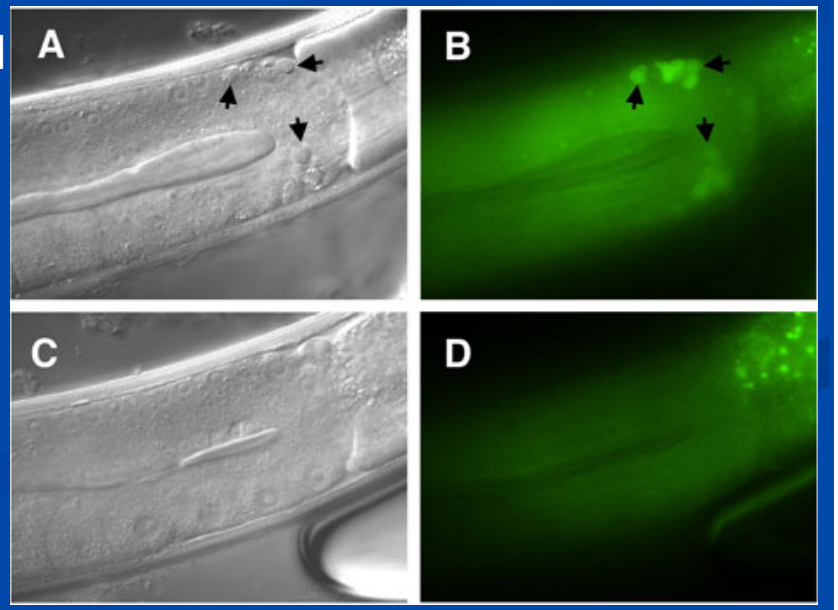


"Death Zone"


A.
Pachytene
Transition zone
Mitotic
Death zone
Distal Tip cell
Oocytes
Sperm
Embryo
B.
Sheath cell engulfing apoptotic germ cell

Methods: Phase 2

1. Select (“pick”) jr431 AD worms in L4 stage.
2. Allow to grow overnight to “Young Adult” stage.
3. Soak worms in SYTO-12 (staining) solution.
4. Transfer approximately 30-40 worms to new plates
5. Heat shock each plate for various increments of time 0,10,20,30 and 60 min.
6. Incubate worms at 20º C for 2 hrs. to recover and purge SYTO-12.
7. Mount worms from each plate on agarose pad
8. Observe worms using the fluorescent component of microscope for detecting dead cells in the “death zone” / gonad area.



Results: Phase 2

- We tried varying amounts of SYTO-12 but were unable to generate any conclusive data.
- A variety of factors would have to be tested to determine how fluorescent marking could be used to accurately determine that the CED-4 gene is actively participating in apoptosis in the gonad region.
- (Variables: feeding time, recovery time, etc.)

How can we identify a mutant? (ex: *ced-4*)

Two Methods

1. Based on phenotype:

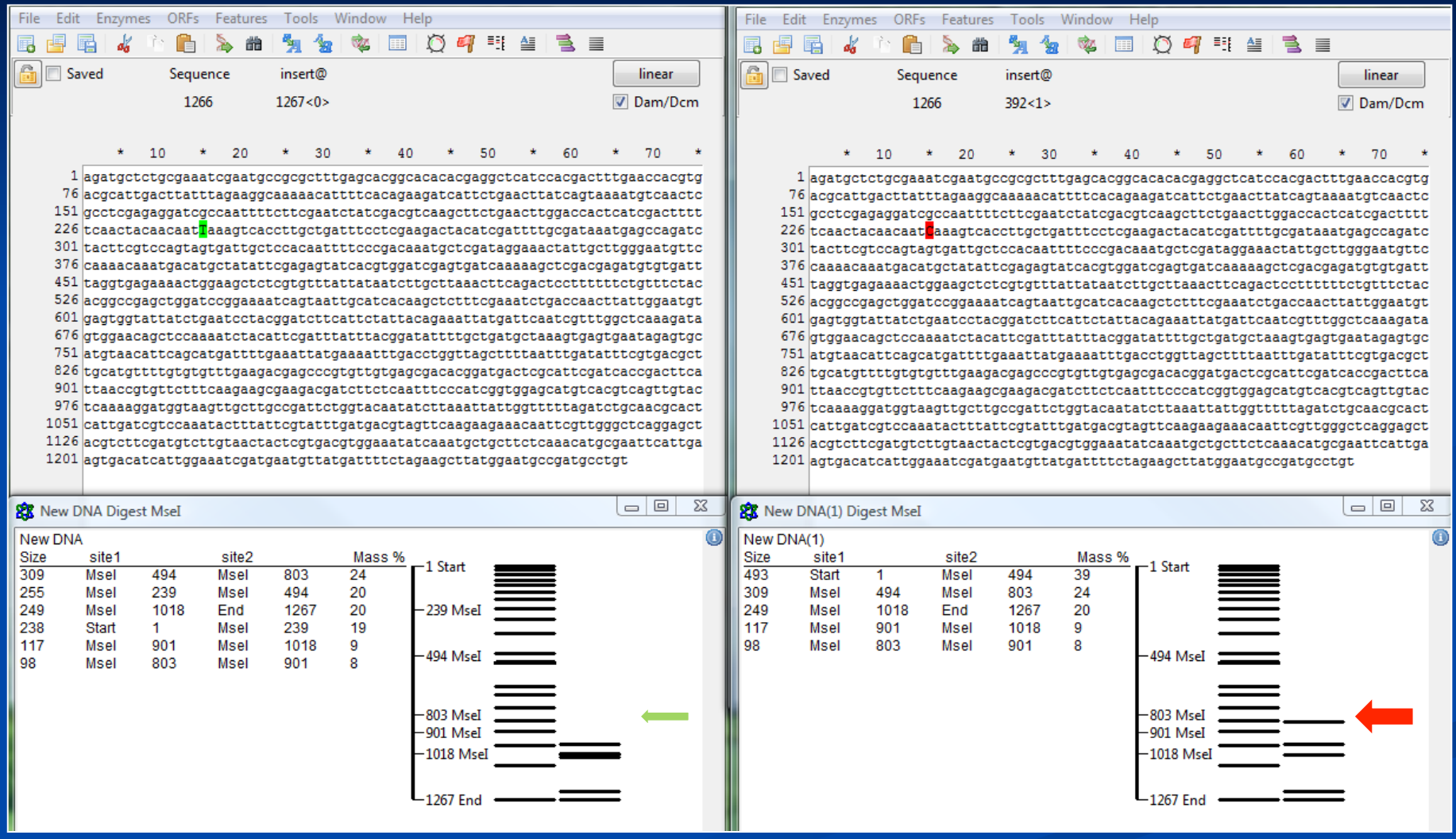
   WT embryo : cell corpse
   ced-4 : no cell corpse

2. Based on DNA sequence

   A. Simple method: by restriction enzyme digestion
   B. DNA sequencing

Wild Type

ced-4 mutant

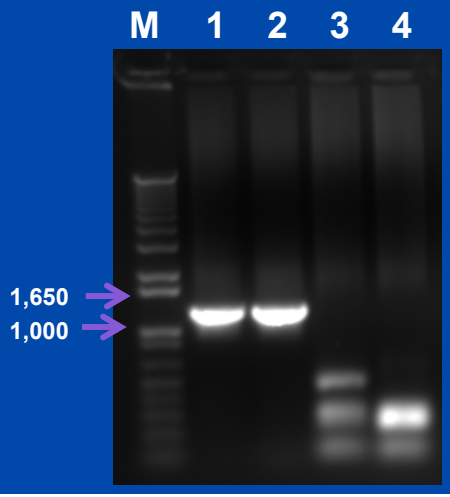


Restriction enzyme site of Mse I

T↓TAA
AAT↑T

PCR product of *ced-4* (1.266bp) in WT

PCR product of *ced-4* (1.266bp) in *ced-4(n1162)*



M : DNA size marker
1: N2 PCR product
2: ced-4 (n1162) PCR product
3: Mse I in 2
4: Mse I in 1

Conclusions & Next Steps

- We were successful in activating the CED-4 function using the Heat Shock treatment.

- Using restriction enzyme digestion we also confirmed that the *ced-4*(-);RNAi mutant fertile phenotype Dr. Jeong will be using is correctly identified.

- Dr. Jeong will continue studying the identification of new functions for CED-4 in embryogenesis and as a cell cycle "checkpoint".

Acknowledgements

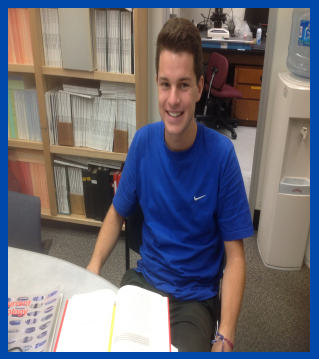
Many thanks to my mentor, co-interns, our lab manager for their help and to the Principal Investigator, for making the lab possible

Mentor

Dr. Pan Young Jeong

RET Supervisor

Dr. Frank Kinnaman

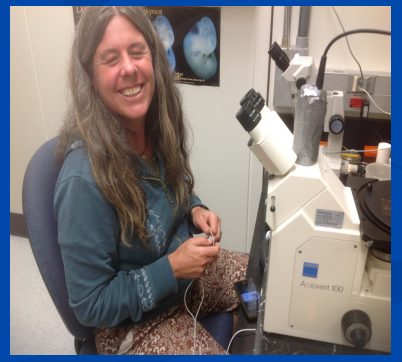


Intern

Dan Roman

Intern

Ame Thakrar



Lab Manager

Cricket Wood

Principal Investigator

Joel Rothman