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

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

- **CED-9**
- **CED-4**
- **CED-3**
- **Mitochondrion**
- **EGL-1**
- **CED-9 inactivation**
- **CED-4**
- **CED-4**
- **CED-3**

**Cell Death**

- **Cancer**
- **Genetic birth disorders**
- **Parkinson’s disease**

*PCD model in C. elegans* 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)

**B. Drosophila**

- Apoptotic stimuli
  - Reaper (Mitochondrion)
  - Grim
  - Hid (IAP antagonists)
  - DIAP1 (Inhibitor of apoptosis)
  - Dronc (Caspase-9-like)
  - P35 (Apafl-1 related killer)
  - DriCE (Caspase-3-like)

**C. Mammals**

- Apoptotic stimuli
  - Bcl-2 (Family members)
  - HtrA2 (SMAC)
  - Cyto C
  - ARTS (IAP antagonists)
  - 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
No Heat

- ced-4(-);pHS::CED-4::FLAG

Heat

- ced-4(-);pHS::CED-4::FLAG

Cell corpse

 jr 431

 jr 431
Methods: Phase 1

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

# Cell Corpses per Comma Cell Embryo

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

- TTAA

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

- TCaa

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”.
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Mentor
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Intern
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Ame Thakrar

Lab Manager
Cricket Wood

Principal Investigator
Joel Rothman

RET Supervisor
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