Survival Rate of Starved *C. elegans*

Kimberly Fletcher, Dr. Lynn Boyd

University of Alabama in Huntsville, Department of Biological Sciences

Research/Creative Experience for Undergraduates

Abstract

The purpose of this experiment is to observe lifespan changes in starved *Caenorhabditis elegans* as compared to those that have a constant food supply. More specifically, this experiment is designed to also show this same comparison between worms that have been treated with RNAi (RNA interference). RNAi knockdown of ubiquitin conjugating enzymes (UBCs) were performed on the organisms starting at the first moment of life. HT115 *E. coli* containing the gene of interest was fed to the worms and *pL4440* (an empty vector) was used as the control. The point of knocking down UBCs in *C. elegans* is to understand if inhibiting ubiquitin linkage to the targeted protein will prolong or shorten the life of the worm. RNAi of *ubc-2* was the only experiment that showed starved worms living longer than nourished worms. Overall, fed worms either lived just as long or longer than starved worms.

Introduction

When an organism is starved, the cell's non-vital areas of the body are broken down to supply nutrients to the vital parts. The Ubiquitin pathway has been shown to play a part in this decomposition. UBCs are also called E2s. There are a bout thirty of these enzymes that perform the second step in the Ubiquitin system.

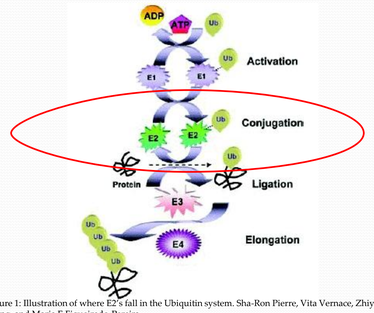
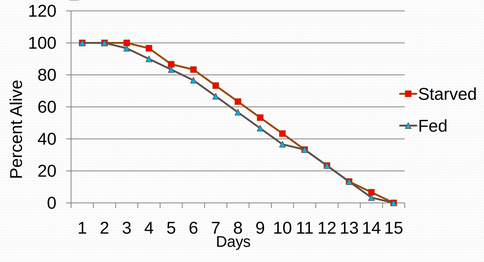


Figure 1: Illustration of where E2's fall in the Ubiquitin system. Sha-Ron Pierre, Vita Vernace, Zhiyou Wang, and Maria E Figueiredo-Pereira
Assembly of Protein Aggregates in Neurodegeneration: Mechanisms Linking the Ubiquitin/Proteasome Pathway and Chaperones

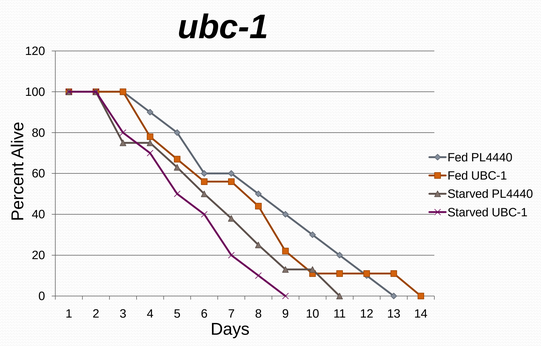
RNAi is very important for analyzing the Ubiqitin system. The way RNAi works is the gene that is being targeted for knockdown is put into the plasmid of the bacteria. When the worms digest the food, they also digest the gene. The duplication of this gene in the worm's cell causes all of the genes of that type to be destroyed, leaving no gene to be expressed.

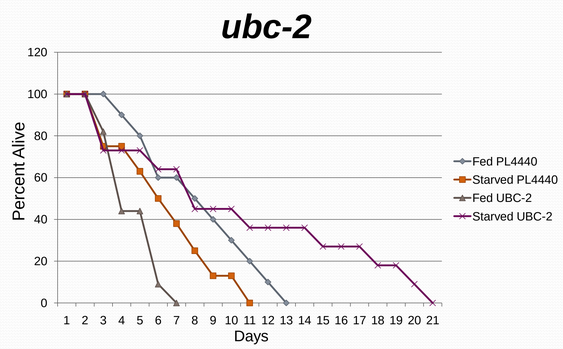
Background

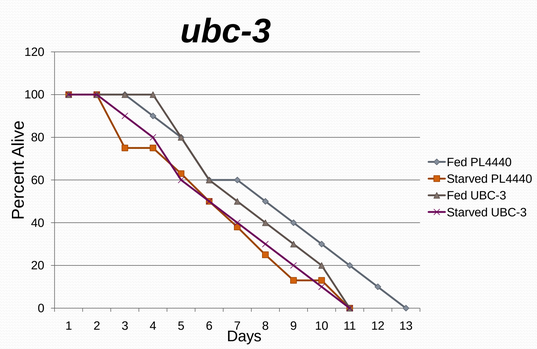
The work completed on this project prior to the RNAi procedure was a simple comparison of fed and starved wild type worms with OP50 *E. coli* bacteria. Three total trials were done and averaged into the figure to the right.

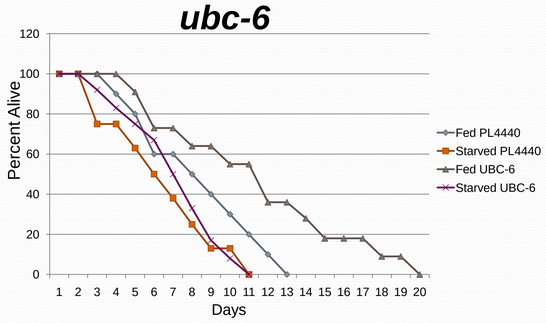


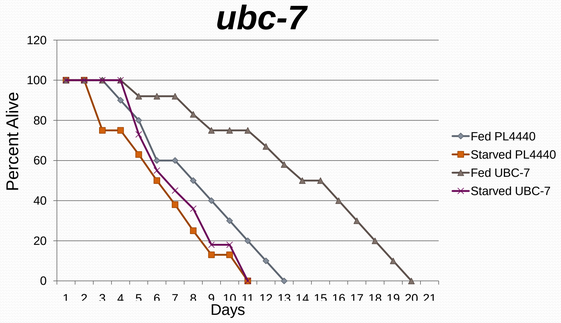
Results

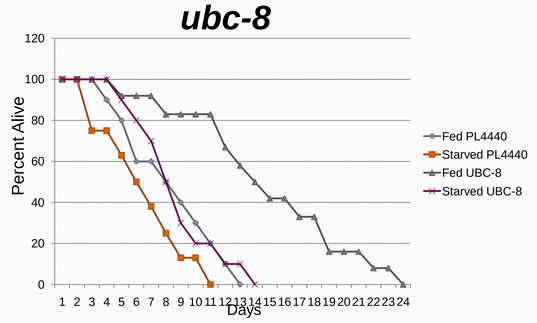


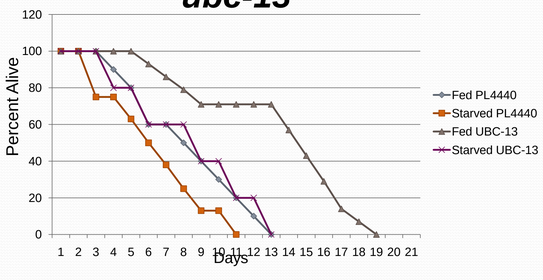


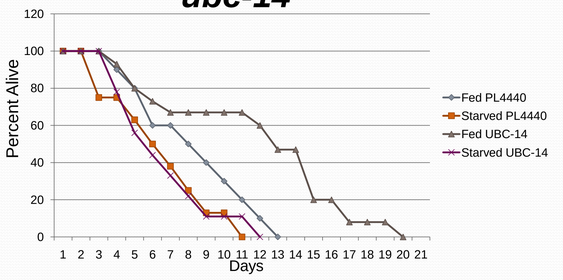


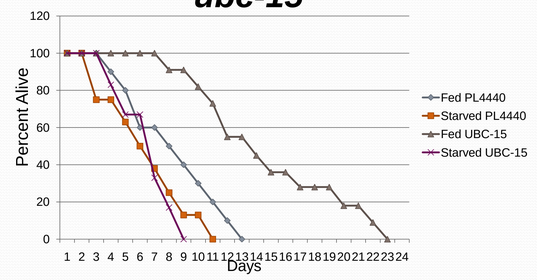


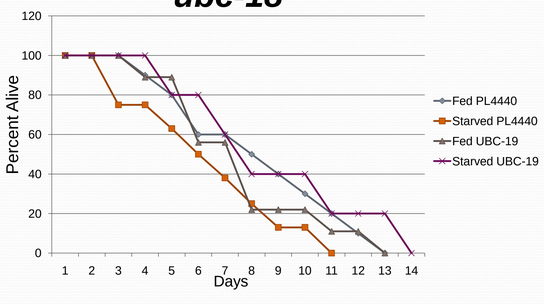


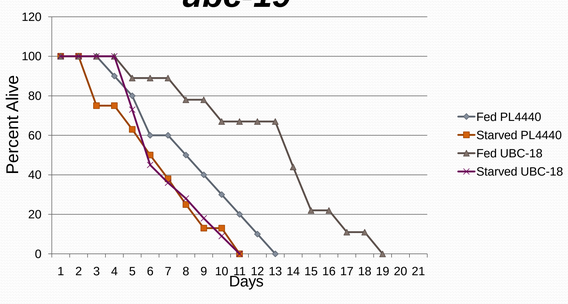


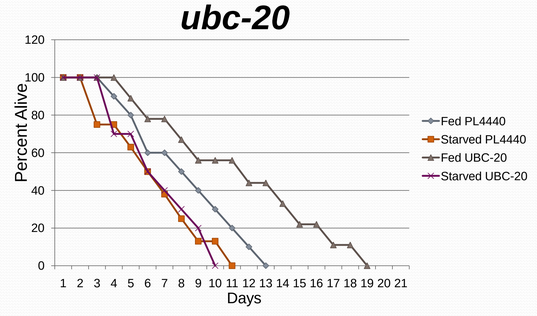












Conclusions and Future Research

- Overall, C. elegans treated with RNAi lived longer under nourished conditions.
- *ubc-2* was the only enzyme that showed to prolong the life of the worms under starvation environment when compared to the fed environment. This could mean *ubc-2* prohibits the breakdown of vital tissue during starvation.
- In the future, the experiments will be repeated and compared to this trial. Also, *ubc-21* through *ubc-26* will be tested in the same manner as the rest of the E2s.

Methods

Wild type (N2) *C. elegans* were used for this experiment. The worms were gown to the adult stage (when the eggs are still in the parent worm) in a 20 C incubator. The worms were broken open using a mixture of bleach, water, and 1 M sodium hypochlorite. The worm eggs were then put on agar plates that contains a specific RNAi bacteria. The worms consume the bacteria and grow to a young adult stage (known as L4). From this stage, the worms are washed using M9 buffer. About a dozen worms are put back on the RNAi bacteria they grew up on and a dozen are put on agar plates that contain Ampicillin, Kanamycin, and Gentamycin. From this day, the worms are checked and scored everyday at the same time. The worms are scored as either dead, alive, or missing. When all of the worms are off of the plate, a survival curve was made using how many are alive divided by the total of worms that started on the plate times one hundred.

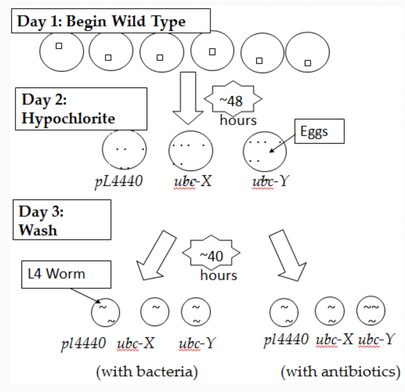


Figure 2: A graphic overview of the overall "Fed vs. Starve" oricess. This is my own work.

Acknowledgements

- UAH 2011 RCEU Program
- Dr. Bernhard Vogler, for his efforts in organizing the RCEU program
- Dr. Lynn Boyd, for allowing the opportunity to perform this study
- Alabama Space Grant Consortium
- President's office of UAHuntsville
- Department of Chemistry