The Role of RPN-12, a Subunit of the Proteasome's 19S Regulatory Particle, in *C. elegans* Male Fertility

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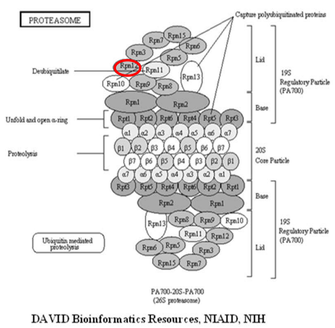
ABSTRACT

The proteasome is a highly conserved protein complex consisting of nearly 33 different subunits of interacting proteins, that degrades proteins and maintains protein homeostasis in cells. The proteasome consists of a 19S regulatory particle (RP) lid and base component capping a cylindrical 20S core particle (CP). Our research investigates roles for individual 19S RP proteasomal subunits in fertility using the model organism, *Caenorhabditis elegans*. My project focuses on characterizing the role of the 19S RP subunit RPN-12 in *C. elegans'* male fertility. Our lab has shown that in *C. elegans,* the endogenous *rpn-12* deletion mutant (*rpn-12(av93)* causes feminization of the hermaphrodite germ line. Surprisingly, the male *rpn-12(av93)* animals show no apparent defects in growth, survival, or fertility. To investigate the role of RPN-12 in *C. elegans* males, I quantify *rpn-12(av93)* male spermatids and perform fertility assays to assess the ability of the males to copulate and successfully fertilize oocytes. There is no difference in average brood of *rpn-12(av93)* males mated to females compared to wildtype(N2) males. However, a high percentage of males run off the agar and dry on the walls of the plate. Therefore, we hypothesize that while RPN-12 may not be necessary for sperm production in *C. elegans* males, it may be essential for sensing the presence of the female and copulation. Future experiments will be performed to assess the male mating behavior of *rpn-12(av93)* mutant. Collectively our data provides further support that specific proteasome subunits perform different, non-proteolytic, functions in specific tissues.

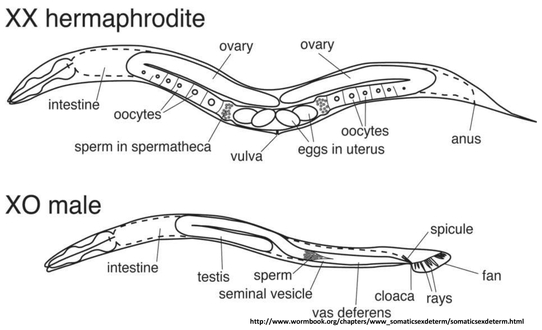
BACKGROUND

RPN-12 is a 19S RP lid subunit of the 26S proteasome



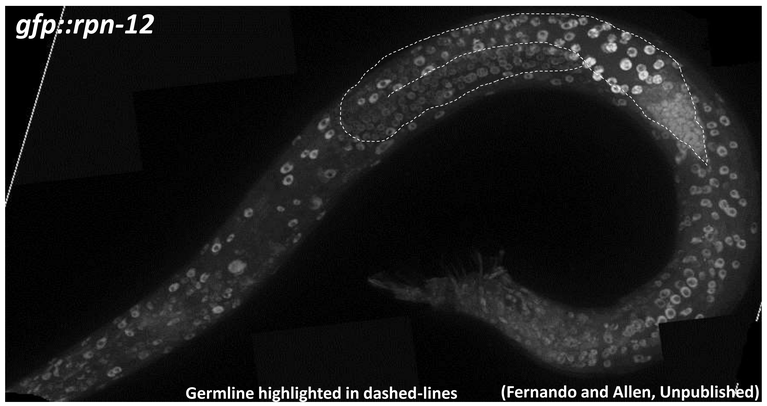
DAVID Bioinformatics Resources, NIAID, NIH

***C. elegans* germline anatomy of males and hermaphrodites**



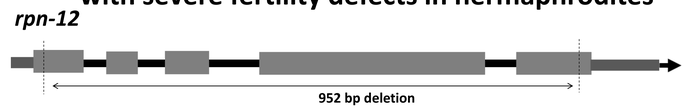
http://www.wormbook.org/chapters/www_somaticsexdeterm/somaticsexdeterm.html

RPN-12 is expressed in both germline and soma of *C. elegans* males

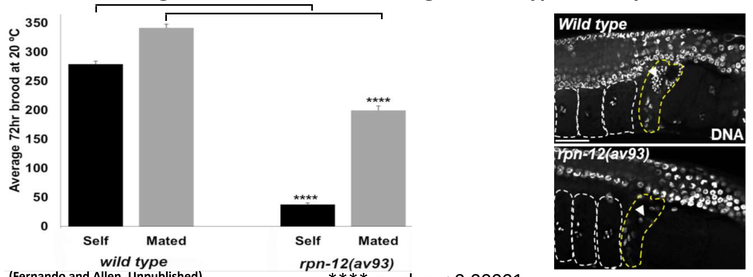


BACKGROUND

A loss of function mutant *rpn-12(av93)* is homozygous viable with severe fertility defects in hermaphrodites



***rpn-12(av93)* mutant hermaphrodites lack sperm but can be rescued by mating however, animals do not gain wild-type fertility**



(Fernando and Allen, Unpublished)

**** *p* value < 0.00001

OBJECTIVE

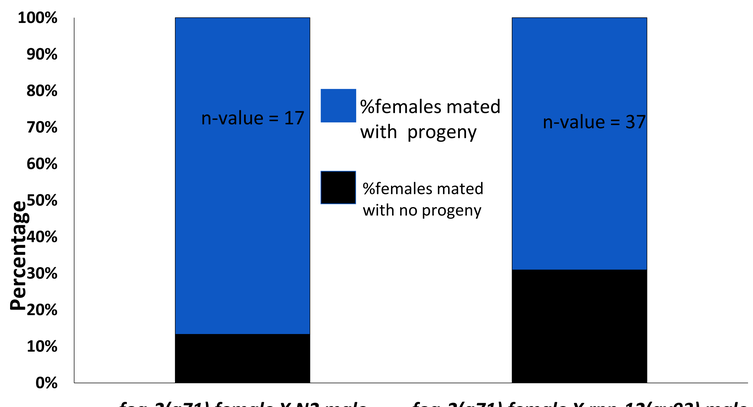
To assess if there are fertility and/or copulation defects in *rpn12(av93)* mutant males.

METHODS

Age synchronized wild type and *rpn-12(av93)* males were mated with phenotypically female *fog-2* animals in a 1: 1 ratio. The worms were observed for 72 hours and the progeny produced by each mating pair was scored.

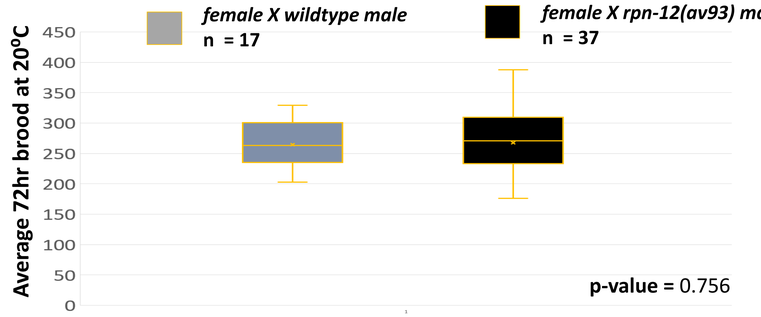
RESULTS

Mating feminized animals with *rpn-12(av93)* males is less likely to result in progeny

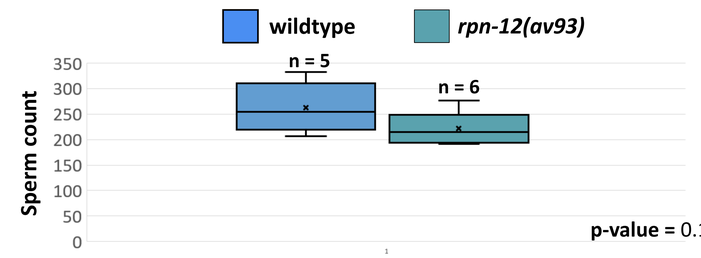


RESULTS

Loss of RPN-12 does not affect the average brood size of males who successfully copulated



***rpn-12(av93)* males have a slight reduction in spermatid number that is not statistically significant**



CONCLUSION

Loss of RPN-12 in males does not severely affect viability or sperm count but may play a role in successful copulation.

FUTURE DIRECTIONS

- **Determine whether male worms experience difficulty in sensing the presence of the female or exhibit different mating behavior from wildtype males**
- **Determine whether sperm is present in females with no progeny using Mito Tracker fluorescent dye**
- **Record mating behavior of *rpn-12(av93)* male worms**
- **Determine if there are any defects in the male tale using DIC imaging**

REFERENCES

1. Boehringer, J., Riedinger, C., Paraskevopoulos, K., Johnson, E. O., Lowe, E. D., Khoudian, C., … Endicott, J. A. (2012). Structural and functional characterization of Rpn12 identifies residues required for Rpn10 proteasome incorporation. The Biochemical journal, 448(1), 55–65. doi:10.1042/BJ20120542

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