Role of proteasome subunit RPN-12 in *C. elegans* germline sex determination and oogenesis

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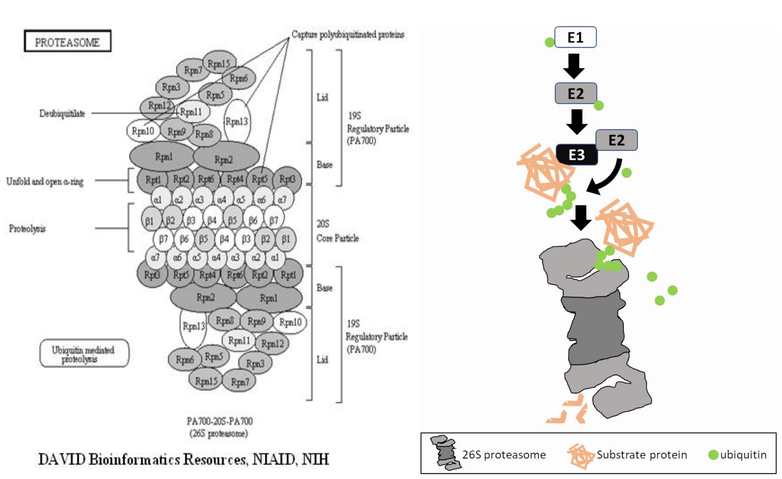
ABSTRACT

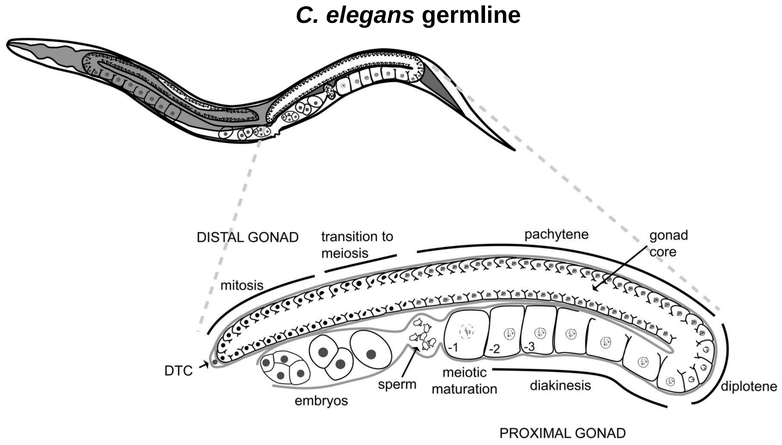
The 26S proteasome is a major proteolytic machinery that is conserved across species. Proper function of the proteasome is crucial for the survival of organisms. The proteasome is composed of nearly 33 different subunits arranged into two 19S regulatory particles (RP) capping a cylindrical 20S core particle (CP). Recent evidence suggests that specific 19S RP subunits of the proteasome perform non-proteolytic roles in various cellular processes such as transcription, mRNA export and chromatin structure. However, there is still a large knowledge gap in our understanding of specific canonical and non-canonical roles of each proteasome subunit. In *Caenorhabditis elegans,* depletion of individual proteasome subunits typically results in embryonic lethality and thus elucidating explicit roles of individual subunits is challenging. We recently found that the 19S RP subunit RPN-12 is not essential for the general proteolytic function of the proteasome but for hermaphrodite gametogenesis in *C. elegans*. A null mutant of RPN-12, *rpn-12(av93),* is homozygous viable, however the hermaphrodites lack sperm. The oocytes of the *rpn-12(av93)* animals can be cross fertilized by mating with males but the hermaphrodites do not reach wild-type reproductive capacity. Therefore, we hypothesized that RPN-12 plays a role in *C. elegans* germline sex determination pathway and oocyte quality respectively. Loss of RPN-12 causes nuclear accumulation of the oocyte meiotic kinase WEE-1.3 which is essential for oocyte quality and *rpn-12(av93)* hermaphrodites can partially suppress the *wee-1.3(RNAi)* infertility phenotype. As chemical inhibition of the proteasome using bortezomib neither causes WEE-1.3 nuclear accumulation in oocytes nor suppresses the *wee-1.3(RNAi)* infertility phenotype, this suggests a potential non-proteolytic role for RPN-12 in WEE-1.3 regulation. Current investigations are underway to identify the mechanism by which RPN-12 regulates the germline sex determination pathway and its interaction with WEE-1.3 in maintaining oocyte quality. To further characterize RPN-12 we generated an endogenous GFP::RPN-12 tagged strain which shows ubiquitous expression in the germline and somatic cells. The *gfp::rpn-12* strain will be used to perform *in vitro* and *in vivo* studies to identify interactors of RPN-12 and further determines its non-proteolytic roles in the *C. elegans* germline.

BACKGROUND

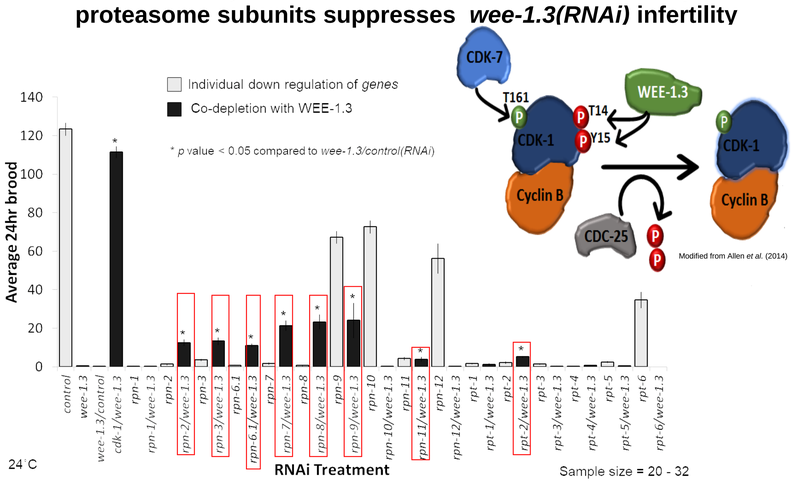
26S proteasome is a highly conserved proteolytic machinery in cells



C. elegans germline

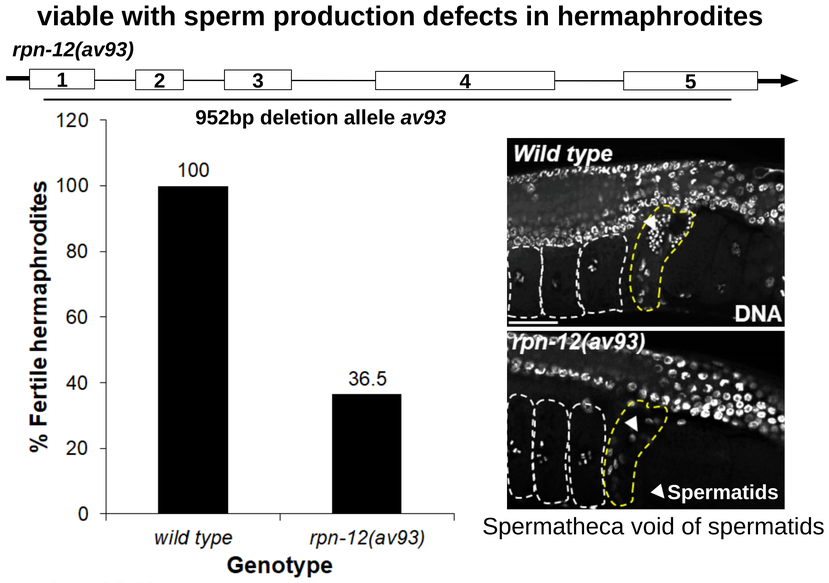


Myt1 ortholog in *C. elegans*, WEE-1.3 is a major inhibitory kinase involved in oocyte maturation and down regulation of specific proteasome subunits suppresses *wee-1.3(RNAi)* infertility



RESULTS

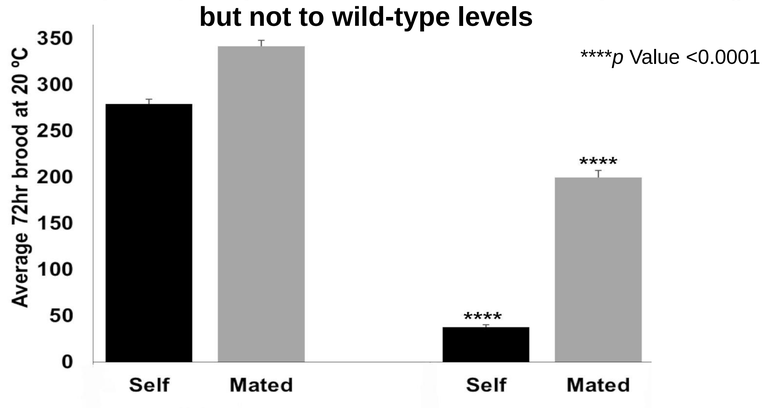
RPN-12 complete loss of function mutant is homozygous viable with sperm production defects in hermaphrodites



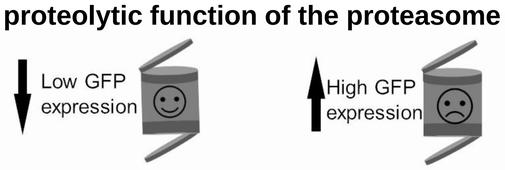
Spermatheca void of spermatids

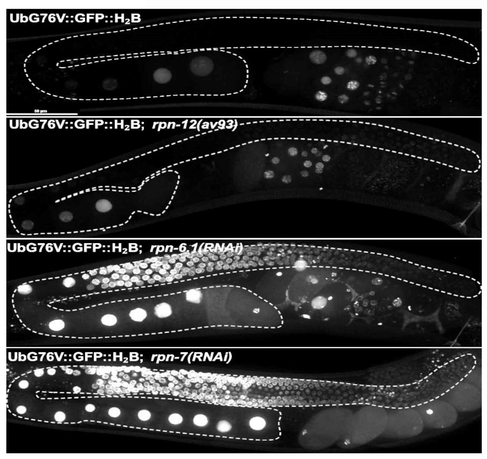
Only 36.5% hermaphrodites were self fertile

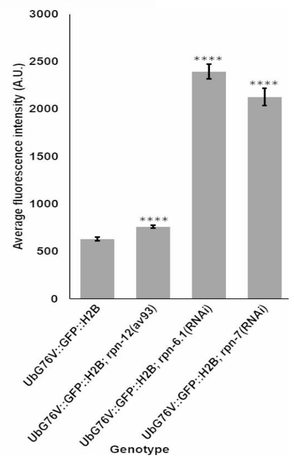
Infertile *rpn-12(av93)* mutants can be rescued upon mating but not to wild-type levels



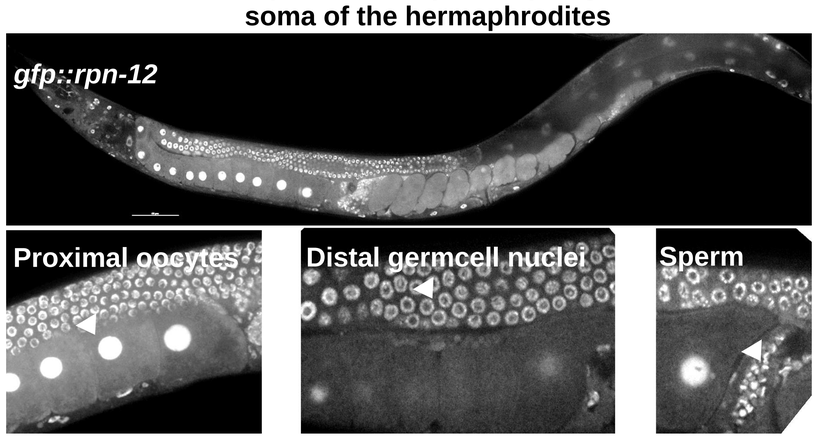
Loss of RPN-12 causes minor impairment of the proteolytic function of the proteasome





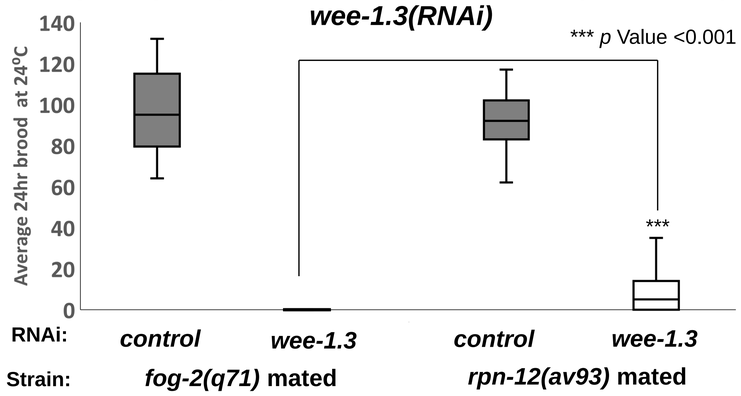


RPN-12 is expressed ubiquitously in germline and soma of the hermaphrodites

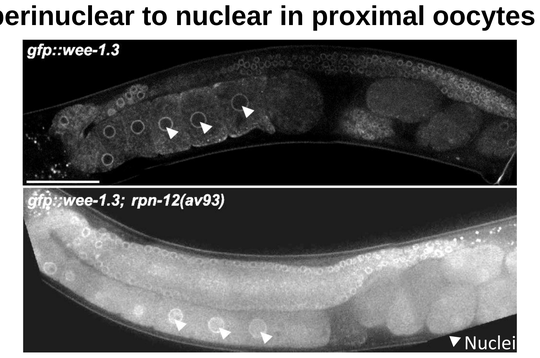


RESULTS

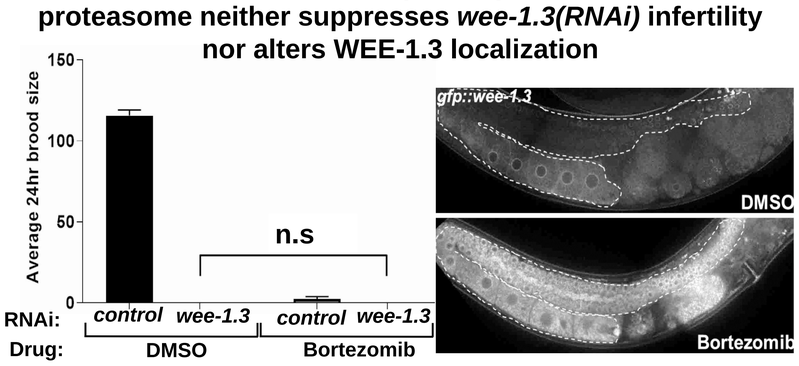
rpn-12(av93) mutants suppress infertility caused by *wee-1.3(RNAi)*



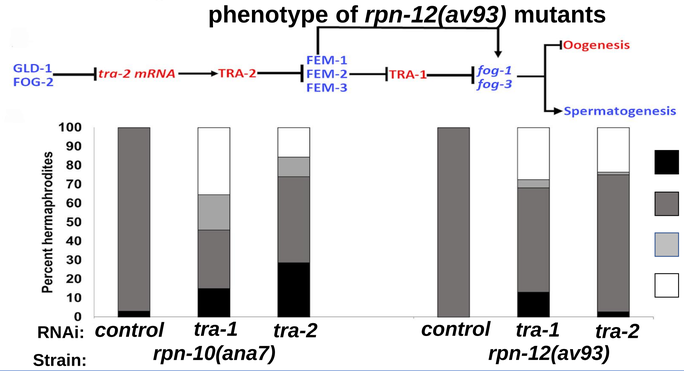
Loss of RPN-12 alters localization of WEE-1.3 from perinuclear to nuclear in proximal oocytes



Chemical inhibition of the proteolytic function of the proteasome neither suppresses *wee-1.3(RNAi)* infertility nor alters WEE-1.3 localization



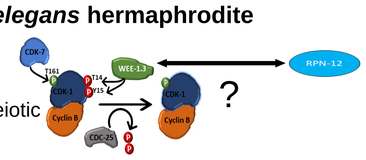
Down regulation of TRA-1 via RNAi suppresses lack of sperm phenotype of *rpn-12(av93)* mutants



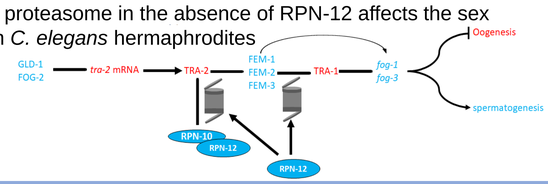
SUMMARY

RPN-12 plays an essential role in *C. elegans* hermaphrodite reproduction

- RPN-12 may play a previously unknown proteolytic or non-proteolytic role in oocyte meiotic maturation pathway



- Partial impairment of the proteasome in the absence of RPN-12 affects the sex determination pathway in *C. elegans* hermaphrodites



ACKNOWLEDGEMENTS

- Members of the Allen Lab
- Golden Lab at NIH
- Other labs in the department of biology
- Funding sources

Just-Julian Graduate Assistantship