Functional analysis of RPN-13, a *C. elegans* proteasome subunit, using a strain that indicates proteolytic function in the germline

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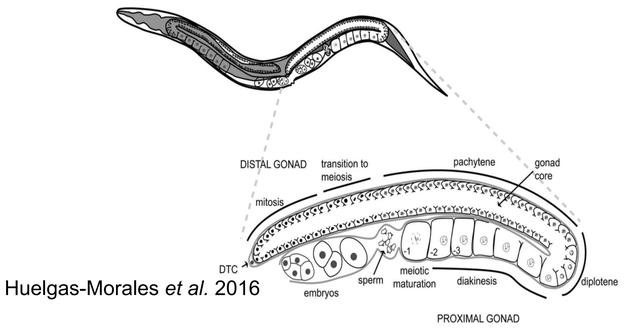
Abstract

The 26S proteasome degrades proteins tagged with ubiquitin. It is composed of a cylindrical 20S core particle (CP) capped with two 19S regulatory particles (RP). The 19S RP is composed of many subunits, and in many cases the specific proteolytic and non-proteolytic roles of individual 19S RP subunits are not fully understood. Our lab identified that certain 19S RP subunits appear to play non-proteolytic roles in the *C. elegans* germline. To learn more about the function of particular 19S RP subunits, we are using a recently published strain, IT1877, to monitor proteolytic activity of the 26S proteasome under various conditions, such as RNAi depletion of different 19S RP subunits. The IT1877 strain contains a mutated ubiquitin fused to GFP under control of the germline promoter [*pie-1p*::Ub(G76V)::GFP::H2B::*drp-1* 3'UTR; *unc-119(+)*] and results in continuous degradation of GFP during optimal 26S proteasome activity. If a certain condition causes proteasome dysfunction, the degree of dysfunction can be quantified by measuring the intensity of the GFP signal in the IT1877 strain. A strong GFP signal indicates 26S proteasome dysfunction, and a dim or no signal indicates the proteasome is functioning properly. As our lab is interested in utilizing the IT1877 strain to monitor proteasome function upon depletion of genes that potentially affect the fertility of the animals, we wanted to determine if the IT1877 strain itself had any fertility defects. A fertility assay was conducted to compare the average 72-hour brood in IT1877 and wild type hermaphrodites. Our data shows that IT1877 hermaphrodites have a significantly lower average brood compared to wild-type animals. Although the IT1877 strain will not be used for future fertility screens in the lab, it will continue to be a useful tool to measure proteolytic activity of the proteasome in the *C. elegans* germline. We are currently using the IT1877 strain to investigate the proteolytic function of the RPN-13 subunit. RPN-13 is an uncharacterized 19S RP subunit in *C. elegans.* Since little is known about this subunit, we will explore its role in *C. elegans* fertility and identify any potential phenotypes observed upon RNAi depletion. Functional analysis of 19S RP subunits, such as RPN-13, will increase our understanding of how the 26S proteasome influences *C. elegans* reproduction.

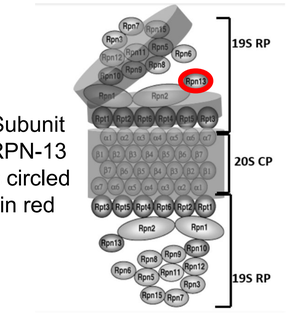
Background

C. elegans hermaphrodite germline



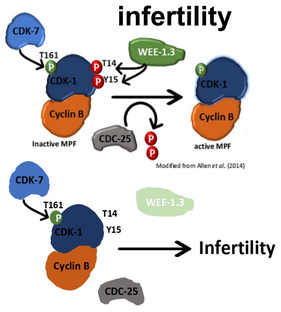
Huelgas-Morales *et al.* 2016

26S Proteasome overview

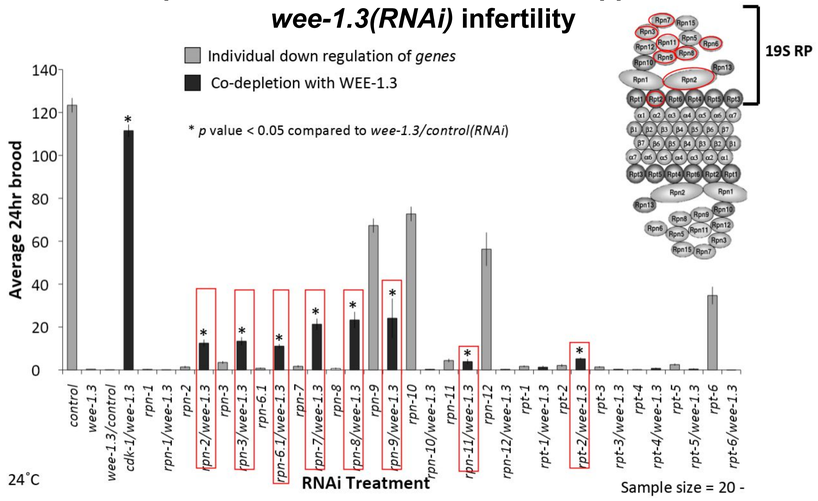


Subunit RPN-13 is circled in red

Downregulation of WEE-1.3 leads to infertility

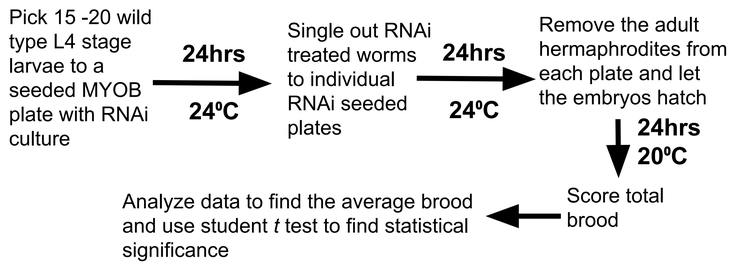


Depletion of certain 19S subunits suppresses *wee-1.3(RNAi)* infertility

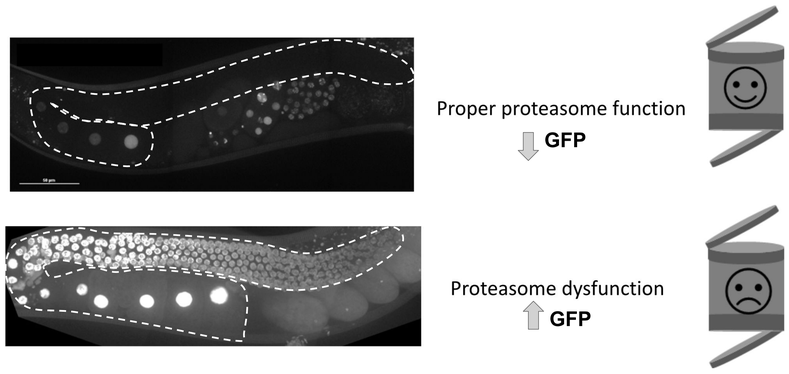


Materials and Methods

RNAi feeding protocol for fertility assay



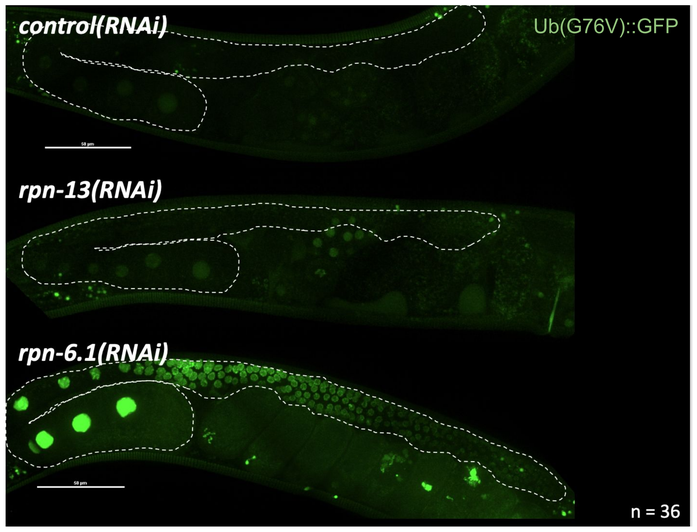
Strain IT1877 monitors proteolytic activity of the proteasome in *C. elegans* germline



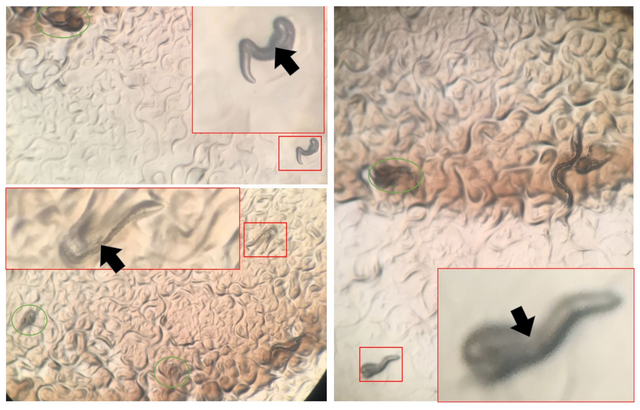
The IT1877 strain contains a mutated ubiquitin fused to GFP under control of the germline promoter [*pie-1p*::Ub(G76V)::GFP::H2B::*drp-1* 3'UTR; *unc-119(+)*]

Results

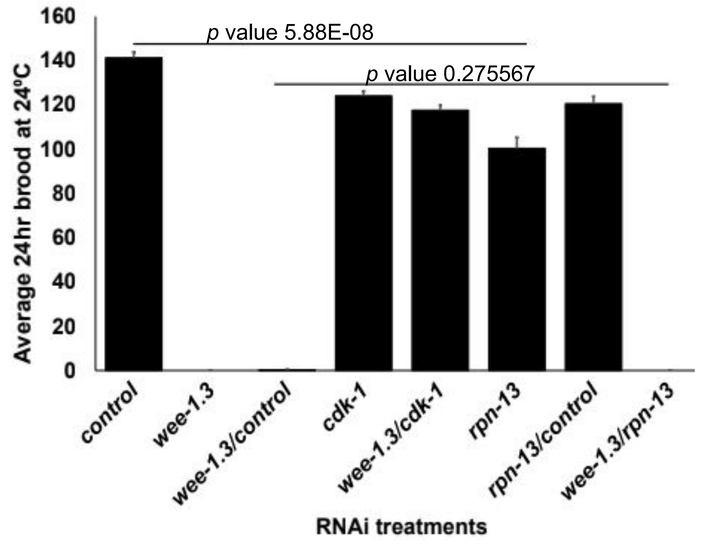
Downregulation of RPN-13 via RNAi does not decrease proteolytic activity of the proteasome in the germline



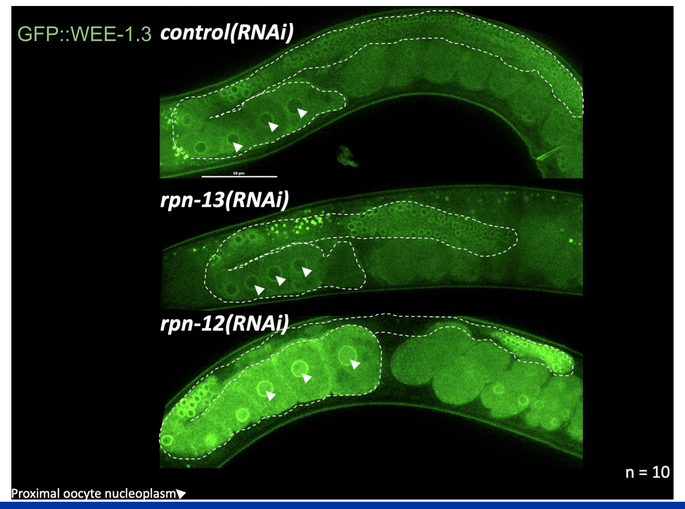
F1 progeny of *rpn-13(RNAi)* parents show ruptured through vulva (Rup) phenotype



Downregulation of RPN-13 via RNAi does not suppress *wee-1.3(RNAi)* infertility



Downregulation of RPN-13 does not alter WEE-1.3 localization



Conclusions

- Progeny of *rpn-13(RNAi)* animals show ruptured vulva phenotype
- Downregulation of RPN-13 does not cause lethality nor does it effect proteasome function in wild type *C. elegans*
- Downregulation of RPN-13 does not suppress *wee-1.3(RNAi)* infertility nor affect WEE-1.3 localization

Future Directions

- Performing *rpn-13(RNAi)* on RNAi sensitive animals, such as *rrf-3* mutants, to see if there is an enhancement of phenotypes observed in wild type animals
- Identify if there are any germline specific roles of RPN-13
- Generating *rpn-13* mutants using CRISPR/Cas9 genome editing technology

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