CHARACTERIZING THE EFFECTS OF *WEE-1.3* KNOCKDOWN ON SPERMATOGENESIS IN *CAENORHABDITIS ELEGANS*

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Honors Degree in Biological Sciences with Distinction

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Meiosis is a specialized type of cell division that results in the production of haploid gametes. This can take the form of oogenesis, which produces oocytes, or spermatogenesis, which produces sperm. In order for meiosis to occur, a protein complex known as maturation promoting factor (MPF) must be activated. In the model organism *Caenorhabditis elegans,* the inhibitory kinase WEE-1.3 regulates meiotic entry by preventing MPF from being activated until the appropriate stage of the cell cycle is reached. Previous work has shown that abnormal WEE-1.3 activity can cause various meiotic defects. Knockdown of *wee-1.3* via RNA interference (RNAi) in hermaphrodites resulted in sterility caused by precocious oocyte maturation. The goal of this project is to characterize the effects of *wee-1.3* knockdown on spermatogenesis in *C. elegans.* Previous to this study, little was known about the role of WEE-1.3 in spermatogenesis except that gain-of-function mutations in the *wee-1.3* gene cause primary spermatocyte arrest during spermatogenesis. I found that knockdown of *wee-1.3* via RNAi was found to negatively impact male fertility and cause severe germline defects. Upon exposure to *wee-1.3* RNAi, some male *C. elegans* completely fail to develop a germline. To further examine the role of *wee-1.3* during spermatogenesis, I plan to use auxin-inducible degradation. Unlike RNAi, auxin-inducible degradation can be used to deplete the target protein in a specific tissue and with relative rapidity. I have generated a *C. elegans* strain that will allow us to deplete WEE-1.3 in the germline.

Chapter 1

INTRODUCTION

1.1 *C. elegans* as a Model Organism

The nematode *Caenorhabditis elegans* is a powerful model organism used for studying various biological processes. These roundworms are particularly well-suited for research purposes for a variety of reasons (Brenner, 1974). *C. elegans* have a short generation time of 3-4 days at 20°C, and they grow to a length of roughly 1 millimeter. Maintaining these animals in the laboratory is simple, as they are kept on petri dishes and feed on OP50 *E. coli.* They are also transparent, which makes it easy to observe their internal structures, such as the gonad. Wild-type *C. elegans* occur in two different sexes: hermaphrodites and males (Figure 1). Hermaphrodites have two X chromosomes, whereas males have only one. The hermaphrodites produce sperm during their final larval stage, which are then stored in a specialized structure called the spermatheca (Figure 1A), before switching to produce oocytes once they reach the young adult stage (Corsi *et al.* 2015). Hermaphrodites are self-fertile, meaning that they can fertilize their own eggs with their stored sperm, but they can also reproduce by mating with males. When mating occurs, the male sperm will outcompete the hermaphrodite's stored sperm (Chu and Shakes, 2013). In the wild, males occur at a low frequency of roughly 0.2%. In a laboratory setting, a variety of techniques can be used to obtain males for experimental purposes. When *C. elegans* are subjected to heat shock, nondisjunction events are more likely to occur in the meiotic cells of the animals' germlines. This can lead to a higher proportion

of male progeny if X chromosome nondisjunction occurs. Specialized strains of worms contain various mutations in genes such as *him-8*, a gene involved in the pairing of homologous X chromosomes, that lead to a high incidence of male progeny in every generation. This project utilizes both methods to obtain males in order to study spermatogenesis. In addition to strains that produce a high incidence of males, the feminized strain *fog-2(oz40)* was also used. These animals do not produce any sperm and must mate with males to produce progeny.

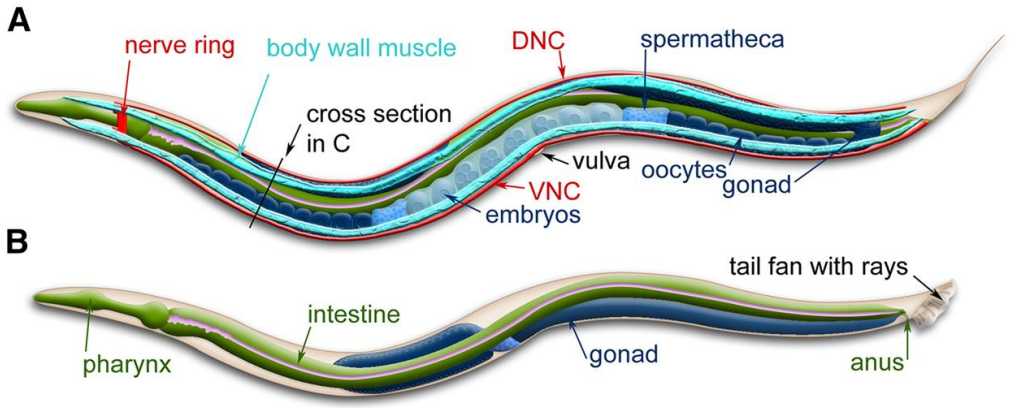

Figure 1.1: Basic *C. elegans* hermaphrodite and male anatomy (Corsi *et al.* 2015). Diagram shows hermaphrodite and male adult *C. elegans* with major organs shown. As depicted in dark blue, hermaphrodites have two, U-shaped gonads (A), while males have a single, J-shaped gonad (B).

1.2 *C. elegans* Reproductive Systems

The male and hermaphrodite *C. elegans* reproductive systems, while structurally similar, differ in a few regards. Hermaphrodites have two U-shaped gonads, whereas males have a single, J-shaped gonad (Figure 1). In both males and hermaphrodites, the distal end of the gonad is capped by the distal tip cell (DTC) and contains mitotic germ cells (Chu and Shakes, 2013). As germ cells move down the germline from the distal tip to the proximal end, they enter the transition zone, followed by meiotic prophase. The

focus of this study is the male reproductive system. In the male germ line, these primary spermatocytes will then undergo both rounds of meiosis to produce four spermatids (Figure 2).

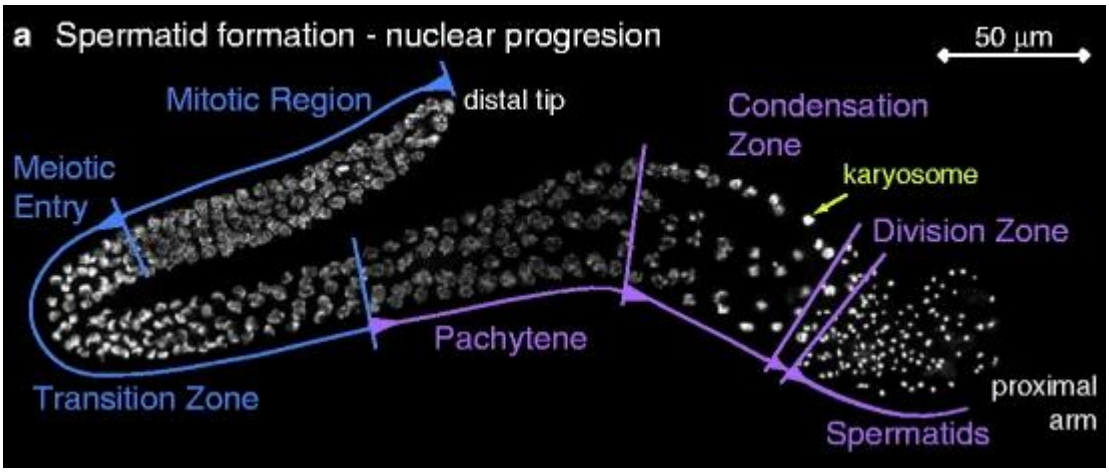

Figure 1.2: Spermatogenesis in *C. elegans* (Chu and Shakes, 2013). Image shows a dissected male gonad with stages of spermatogenesis shown. Starting from the distal tip, germ cells first divide mitotically before entering meiosis. The primary spermatocytes progress through meiosis as they move down the germ line towards the proximal end of the gonad, resulting in the production of spermatids.

1.3 Meiotic and Cell Cycle Regulation

Meiosis is a specialized type of cell division that results in the production of haploid gametes. This is achieved by two subsequent rounds of cell division, termed meiosis I and meiosis II. During meiosis I, homologous chromosomes pair together to be separated. DNA replication does not occur between meiosis I and II. Instead, the sister chromatids are separated from one another during meiosis II. In this way, four haploid daughter cells are produced from one diploid parent cell (Figure 3). Meiosis can take the form of oogenesis, which produces oocytes, or spermatogenesis, which produces sperm.

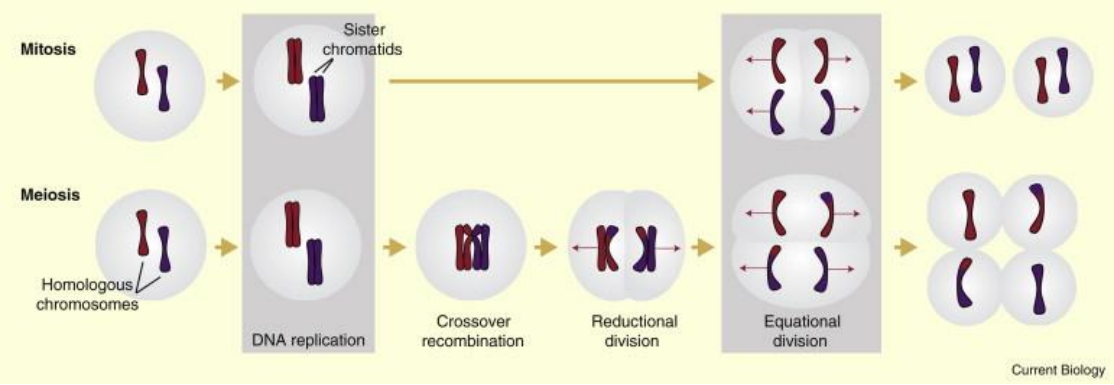

Figure 1.3: Overview of meiosis (Hochwagen 2008). To generate four haploid gametes from one diploid cell, meiosis consists of one round of DNA replication followed by two rounds of cell division.

Considering the complexity of this two-step process, it follows that meiosis must be highly regulated at many levels so that it can proceed correctly. For example, it is essential that a germ cell does not enter meiosis until it is mature enough to do so. For this reason, many checkpoints are present throughout the cell cycle to ensure that a cell is ready to enter the next stage. Progression through the cell cycle is in part driven by the activity of various Cyclin/CDK complexes (Figure 4).

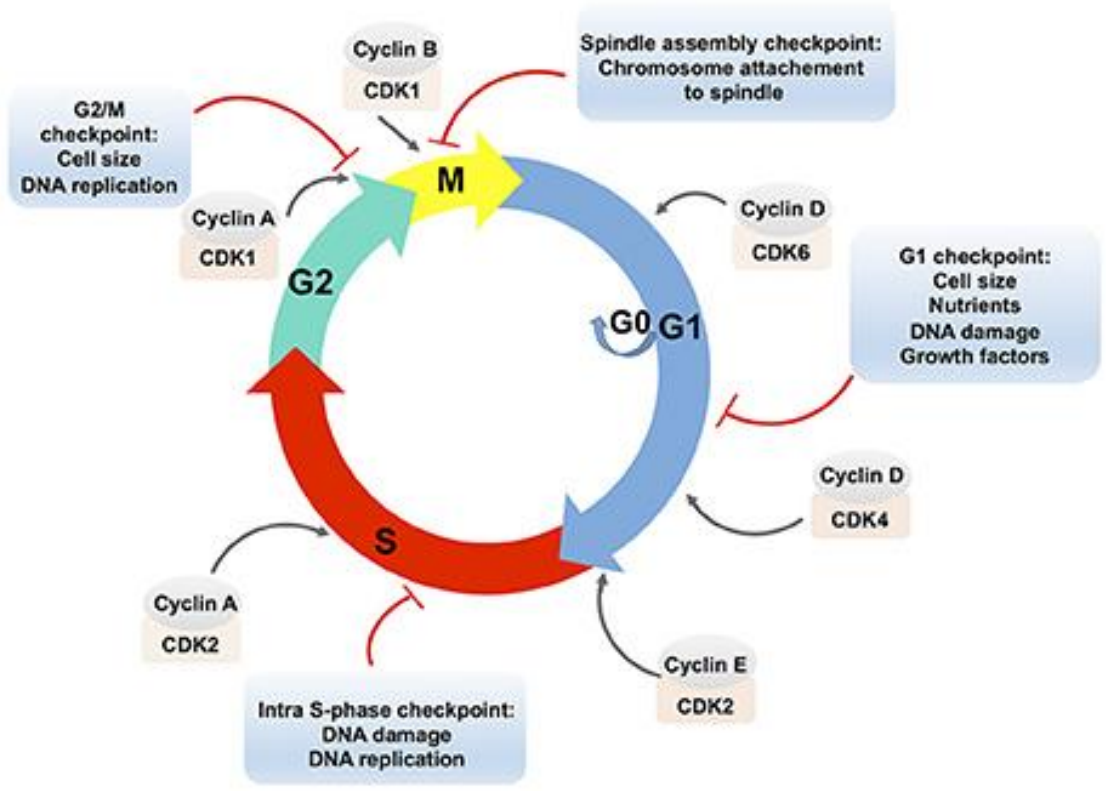

Figure 1.4: Cell cycle regulation (El-Aouar Filho *et al.* 2017). The cell cycle is highly regulated and contains multiple checkpoints, which prevent the cycle from progressing inappropriately. Various cyclin and CDK complexes drive the cell cycle at different points, such as Cyclin B and CDK-1, which drive the transition from G2 phase to cell division, also known as M phase.

In eukaryotes, a protein complex known as Maturation Promoting Factor (MPF) must be activated before germ cells enter meiosis (Lamitina and L'Hernault, 2002). MPF is composed of CDK-1 and its partner Cyclin B. The kinase WEE-1.3 inhibits MPF by phosphorylating CDK-1 at residues Thr14 and Tyr15 (Figure 5). MPF will remain inactive until, among other factors, the phosphatase Cdc25 removes the inhibitory phosphorylations from CDK-1 (Allen et al. 2014).

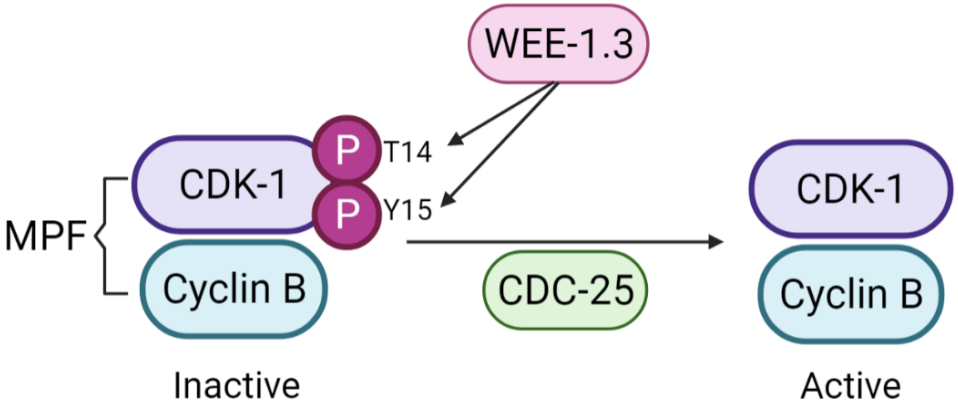

Figure 1.5: The role of WEE-1.3 as a cell cycle regulator. Diagram shows how WEE-1.3 kinase and CDC-25 phosphatase act to regulate maturation promoting factor (MPF) activity. MPF is a complex consisting of CDK-1 (purple) and Cyclin B (blue). The kinase WEE-1.3 (pink) inhibits MPF by phosphorylating CDK-1 at residues T14 and Y15 (magenta). In order for MPF to be activated, CDC-25 phosphatase (green) must dephosphorylate CDK-1 (Allen *et al.* 2014). Image created using BioRender software.

The role of *wee-1.3* has been primarily studied in the context of oogenesis. Previous work has shown that *wee-1.3* knockdown via RNA interference (RNAi) results in infertility caused by precocious oocyte maturation (Burrows et al. 2006). Though highly similar, the processes of oogenesis and spermatogenesis differ from one another in a few regards. One such difference is that spermatocytes proceed immediately from meiotic prophase into meiosis, whereas oocytes undergo a pause during late meiotic prophase I and meiosis only resumes after fertilization. In addition, the process of spermatogenesis does not contain damage checkpoints that are present during oogenesis (Chu and Shakes, 2013). Previous work has suggested that *wee-1.3* has a sperm-specific pathway (Lamitina and L'Hernault, 2002). In this study, several different *wee-1.3* gain-of-function mutations were shown to cause primary spermatocyte arrest during

spermatogenesis but did not affect oogenesis. Little else is known about the role of WEE-1.3 kinase during *C. elegans* spermatogenesis.

1.4 Hypothesis

Based off of what is currently known about the role of WEE-1.3 in *C. elegans* meiosis, I hypothesized that *wee-1.3* knockdown will result in male infertility due to premature meiotic entry during spermatogenesis.

1.5 Specific Aims

1.5.1 Determine the effects of *wee-1.3* knockdown on male meiosis

Wee-1.3 knockdown is known to affect oogenesis by causing premature oocyte maturation and subsequent infertility (Burrows *et al*. 2006). Previous work has suggested that there is a spermatogenesis-specific pathway of *wee-1.3* regulation (Lamitina and L'Hernault 2002), but in general the role of *wee-1.3* in spermatogenesis has not been well-studied. The first aim of this study was to characterize the effects of *wee-1.3* knockdown during spermatogenesis. To do this, two different methods of knockdown were used: RNA interference (RNAi) and auxin-inducible degradation.

RNAi utilizes dsRNA to induce gene silencing (Hannon 2002). When dsRNA for the gene of interest is introduced to *C. elegans*, either by injection or feeding, it binds the target mRNA, leading to mRNA degradation resulting in knockdown of the gene of interest (Hannon 2002). We used RNAi by feeding to knock down *wee-1.3*.

Auxin-inducible degradation is a system that allows for rapid, tissue-specific depletion of a target protein (Zhang *et al.* 2015). A degron tag is added to the target

protein, and in the presence of auxin the plant protein TIR1 will attach to the degron tag and associate with other proteins to form a ubiquitin ligase complex, leading to ubiquitination and subsequent degradation of the target protein (Figure 6) (Ashley *et al.* 2021). For germline-specific depletion in *C. elegans*, the plant protein TIR1 is expressed in the germline under control of the *sun-1* promoter (Zhang *et al.* 2015). Here, we used CRISPR/Cas9 genome editing to insert a C-terminal degron tag at the C-terminal end of *wee-1.3* in a *TIR1 g*ermline strain, with the goal of depleting WEE-1.3 in the male germline.

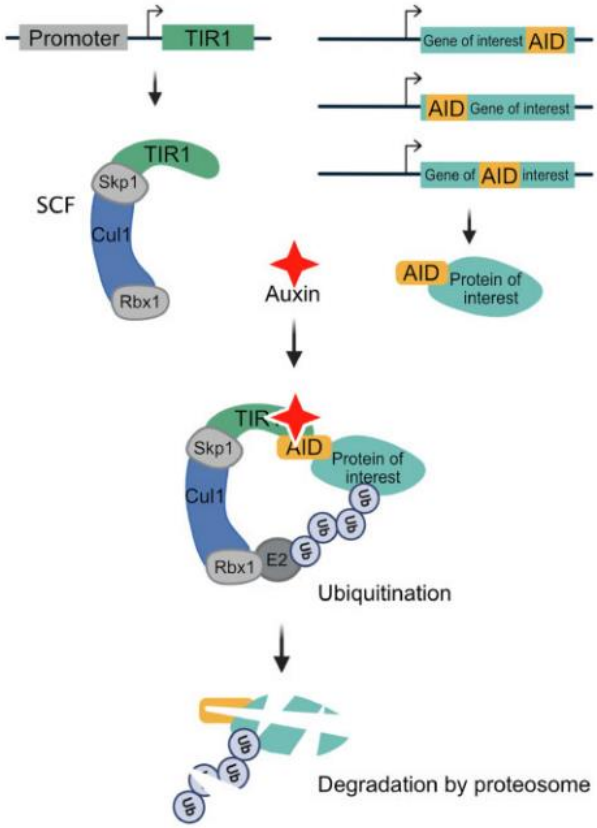

Figure 1.6: Functionality of the auxin-inducible degradation system (Ashley *et al.* 2021). The plant protein TIR1 is expressed in the tissue of interest, and a degron tag is added to the gene of interest. Upon exposure to auxin, TIR1 will form an SCF complex that binds to the degron tag, leading to ubiquitination and subsequent degradation of the protein of interest.

1.5.2 Determine WEE-1.3 localization in male germ lines

Previously, it has been shown that *wee-1.3* is expressed in the soma and germline of adult hermaphrodites (Allen *et al.* 2014; Fernando *et al.* 2021). The second aim of this study was to determine the localization pattern of WEE-1.3 in males.

Chapter 2

METHODS

2.1 *C. elegans* Maintenance and Strains

All strains (Table 1) were maintained using standard technique (Brenner 1974) at 20℃ unless otherwise specified.

Table 1: *C. elegans* strains used for this project

Strain Name	Genotype
N2	Bristol wild-type
WDC2	*wee-1.3(ana2) [gfp::wee-1.3]*
WDC8	*wee-1.3(ana8) [wee-1.3::gfp]*
JDW220	*wrdSi81 (TIR1 germline)*
AJL63	*wee-1.3(ude32) [wee-1.3::aid]*
VC465	*wee-1.3(ok729)/mIn1*
CB14891	*him-8(e1489)*
DG4915	*his-72(uge30); fog-2(oz40)*
AJL69	*wee-1.3(ude32) [wee-1.3::aid]; wrdSi81 (TIR1 germline)*

2.2 RNA Interference by Feeding

For RNAi by feeding (Timmons *et al.* 2001), RNAi plates containing 2 mM IPTG and 25 μg/mL carbenicillin were spotted with *E. coli* expressing dsRNA for *wee-1.3*, *smd-1* (no phenotype, negative control), or *cdk-1* (embryonic lethal, positive control) at least 24 hours before adding worms. 2-4 *him-8(e1489)* L4 hermaphrodites were then

added to each plate and allowed to produce F1 progeny. Once F1 males reached the L4 stage, they were used for further experimentation.

2.3 RNAi Cross, Embryonic Viability Assay, and Brood Size Assay

One *him-8(e1489)* male that was subjected to *wee-1.3* RNAi and one *fog-2(oz40)* female that was not subjected to *wee-1.3* RNAi were each picked onto a single 30 mm MYOB plate spotted with OP50 *E. coli*, for a total of ten plates. They were allowed to mate and lay embryos for 24 hours at 20℃. Every 24 hours, the parentals were picked to a new plate until the mother was done laying embryos. 48 hours after the adults were removed from each respective plate, the plates were counted for larvae and unhatched embryos. For the control, the *fog-2(oz40)* females were mated with *him-8(e1489)* males that were subjected to *smd-1* RNAi using the same protocol. Percent embryonic viability was calculated as the number of larvae divided by the number of total embryos. Brood size was calculated as the total number of dead embryos plus live larvae.

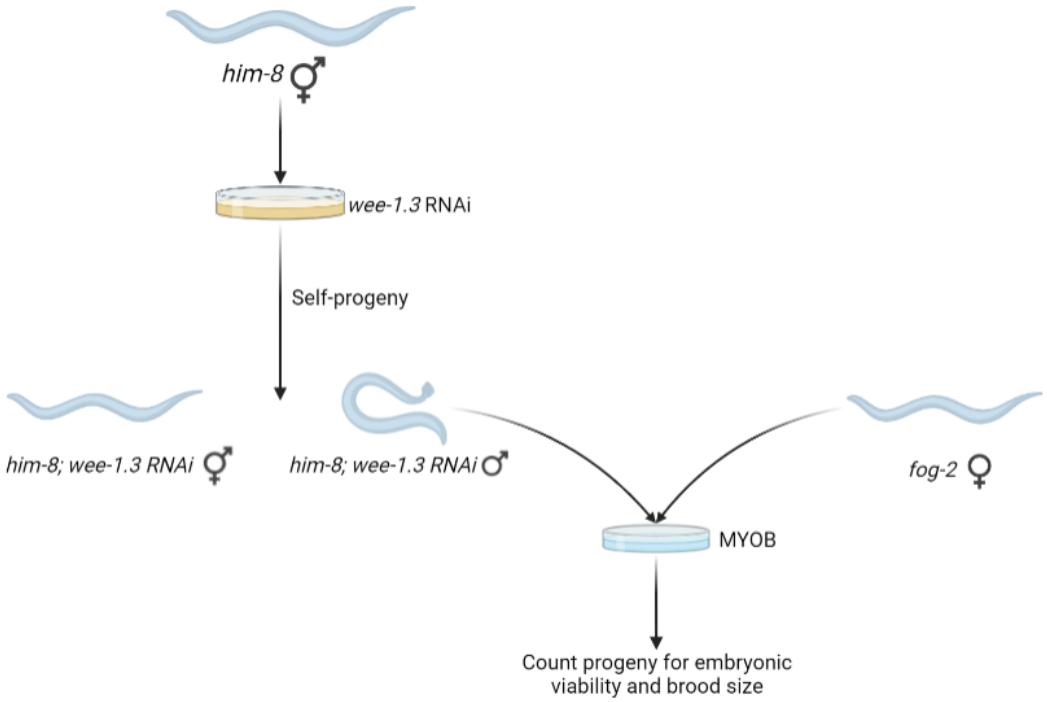

Figure 2.1: RNAi methods. L4 *him-8 (e1489)* hermaphrodites were picked to either *wee-1.3* or control (*cdk-1* or *smd-1)* RNAi plates and allowed to lay progeny. The *him-8 (e1489); wee-1.3* RNAi males were then singled out and picked to individual MYOB plates with one *fog-2 (oz40)* female. Progeny were counted for embryonic viability and brood size.

2.4 Whole-mount DAPI staining

To perform whole-mount DAPI staining, 20 young adult males were picked into 5 µL of M9 on a glass slide. All of the excess M9 was removed from the slide using a Kimwipe before adding 15 µL of 100% room temperature methanol. Once all of the methanol had dried, 12 µL of 2 µg/mL DAPI solution was added to the slide. A coverslip was added and sealed with clear nail polish, and the sample was incubated for 30 minutes in the dark before imaging.

2.5 Gonad Dissections and DAPI staining

Gonad dissections were performed by a paraformaldehyde fixation and freeze/crack with methanol. To do this, 20 individual L4s were picked onto a separate plate 24 hours before dissection. The next day, the adult worms were picked into 30 µL of 1x egg buffer+0.1% Tween on a coverslip supported by a glass slide. Using a surgical blade, the animals were dissected by cutting at the pharynx so that the gonad could be extruded. Once all of the animals had been dissected, 15 µL of the egg buffer solution was removed, and 15 µL of 2% paraformaldehyde solution was added. A Superfrost Plus slide (Fisher Scientific) was then placed on top of the coverslip, and the samples were left to fix for 5 minutes. After 5 minutes, the slide was immersed in liquid nitrogen until all slides had been dissected. Upon removal from the liquid nitrogen, the coverslip was flicked off of each slide (freeze/crack). The slides were then placed in a Coplin jar of -20°C methanol for 1 minute, followed by washing in PBST (1x PBS + 0.1% Tween) for 5 minutes. After this, excess PBST was removed from the slides with a Kimwipe before 40 µL of 2µg/mL DAPI solution was added. The slides were then incubated in the dark for 5 minutes before washing in PBST for 5 minutes in the dark. 8 µL of Vectashield was added to each slide, and a coverslip was placed on top of each slide and sealed with nail polish before imaging.

2.6 Immunostaining

Gonad dissections were performed as described above. After removal from -20°C methanol, the slides were washed 3 times for 5 minutes each in PBST. After the washes, the slides were blocked in a solution of 0.7% BSA in PBST for 1 hour. Excess liquid was then removed from each slide using a Kimwipe, and immunostained with 50 µL of a

1:500 dilution of primary antibody in 0.7% BSA in PBST. A parafilm coverslip was added to each slide before incubating overnight in a dark, humid chamber. The next day, the slides were washed 3 times for 10 minutes each in PBST. Excess liquid was then removed from each slide using a Kimwipe before adding 50 µL of a 1:200 dilution of secondary antibody in 0.7% BSA in PBST. Parafilm coverslips were placed on each slide before incubating for 2 hours in a dark, humid chamber. After the incubation period, the slides were washed 3 times for 10 minutes in PBST in the dark. After this, the slides were DAPI stained. 40 µL of 2 µg/mL DAPI was added to each slide before incubating in the dark for 5 minutes, followed by a 5-minute wash in PBST in the dark. 8 µL of Vectashield was added to each slide, and a coverslip was placed on top of each slide and sealed with nail polish before imaging.

For GFP immunostaining, the antibodies used were rabbit anti-GFP polyclonal (Novus Biologicals, product number NB600308) and anti-rabbit AlexaFluor 568 purchased from Invitrogen (Thermo Fisher Scientific).

2.7 Imaging methods for fixed samples

Initial imaging was performed with a Zeiss AxioObserver microscope. Z-stack images were obtained using a Zeiss LSM780 confocal microscope. Image processing and analysis were conducted using Fiji Is Just ImageJ (Schindelin *et al*. 2012). In order to enhance visualization, contrast and brightness were adjusted.

2.8 CRISPR/Cas9 Methods

To create a system for auxin-inducible degradation of WEE-1.3, CRISPR/Cas9 genome editing was used to insert a degron sequence at the C-terminus of *wee-1.3* using a

dpy-10 co-CRISPR method (Arribere *et al*. 2014). The injection mix consists of Cas9, *dpy-10* CRISPR RNA (crRNA), *dpy-10 (cn64)* repair oligonucleotide, universal trans-activating crRNA (tracrRNA), *wee-1.3 (ude32)* repair oligonucleotide, and crRNA30 (Fernando *et al.* 2021), which targets the C-terminal sequence of *wee-1.3* (5'ATTTGGATCATCAGGCGACGAGG3'). The crRNA guides Cas9 to the cut site, where a double-strand break is generated and then repaired by homologous recombination using the repair template. The *wee-1.3* (*ude32)* repair template used was: (5'tccagATGTCATTTGGATCATCAGGCGACGAGGTT**CCTAAAGATCCAGCCAAACCTCCGGCCAAGGCACAAGTTGTGGGATGGCCACCGGTGAGATCATACCGGAAGAACGTGATGGTTTCCTGCCAAAAATCAAGCGGTGGCCCGGAGGCGGCGGCGTTCGTGAAG**TAAtaatgcacaaaaatcagaaaaattgttgaata3'). The bold region is the degron insertion (Zhang *et al.* 2015). The injection mix was injected into the gonads of either wild-type or *TIR1 germline* hermaphrodites. After the injections, the hermaphrodites were allowed to reproduce, and the F1 generation was screened for the roller (*dpy-10/+*) phenotype. Individual rollers were picked to single plates and allowed to produce self progeny. After ~24 hours of egg laying, the F1 adults were screened for insertion of the degron sequence using PCR and DNA gel electrophoresis. The primers used for PCR were *wee-1.3* forward 2 (5'TTTGGAGCAATCGGAATCGC3') and *wee-1.3* reverse 1 (5'tgaagagatggtcgtcaagg3'). 8-12 progeny from F2 individuals that were heterozygous for the edit were singled out and allowed to lay F3 progeny. Further screening was performed on the F2 generation to identify homozygotes for the edit, again using PCR with the same primers and DNA gel electrophoresis. These results were then sequenced to confirm successful C-terminal degron insertion. Two different strains were

created: AJL63 and AJL69. AJL63 contains the degron insertion in a wild-type background, and AJL69 contains the degron insertion in the *TIR1 germline* background.

2.9 DNA Gel Electrophoresis

Unless otherwise indicated, 2% agarose ethidium bromide gels were used for DNA gel electrophoresis. Gels were run at 100 volts for 90 minutes and imaged using a ChemiDoc (BioRad).

2.10 Live Imaging

To perform live imaging, 5-6 worms were picked into 5 µL of 2 mM tetramisole on a 2% agarose pad. A coverslip was placed on top of each slide before imaging at 20x on a Zeiss AxioObserver microscope.

2.11 Auxin Treatment

Auxin plates were prepared in a similar manner to previously described methods (Zhang et al., 2015). A 400 mM stock solution of the natural auxin indole-3-acetic acid (IAA) (Thermo Scientific) was prepared in 4℃ 100% ethanol and kept at 4℃ for up to one month. Auxin plates were prepared by diluting the auxin solution into 50℃ MYOB media to a concentration of 4 mM before pouring.

Chapter 3

RESULTS

3.1 RNA interference of *wee-1.3* negatively impacts male fertility

To examine how *wee-1.3* knockdown impacts *C. elegans* males, RNAi for *wee-1.3* was performed on *him-8(e1489)* males. These RNAi-treated males were then allowed to mate with *fog-2(oz40)* females that were not treated with RNAi (essentially wild type). Embryonic viability and brood size assays were conducted. While there was no significant difference in average embryonic viability between *wee-1.3* RNAi-treated males and control males (*smd-1* RNAi) (Figure 1A), a significant reduction in average brood size was observed (Figure 1B).

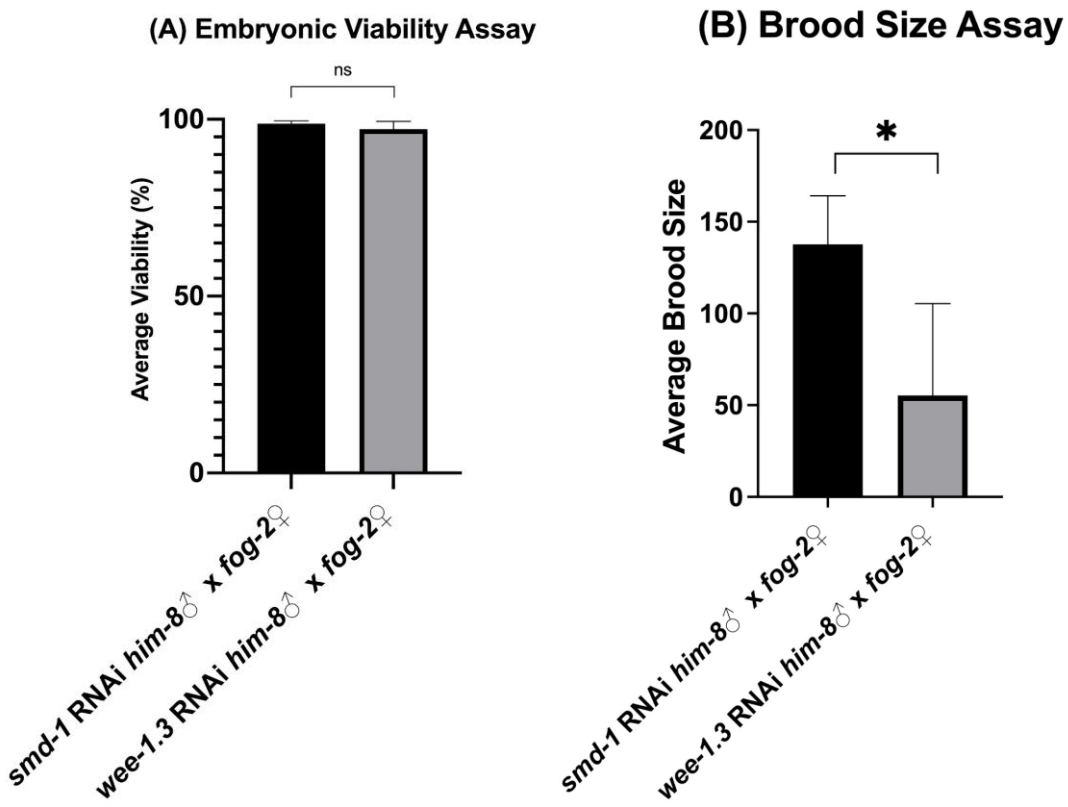

Figure 3.1: Results from crosses between RNAi-treated *him-8(e1489)* males and *fog-2(oz40*) females show that *wee-1.3* RNAi negatively impacts male fertility. (A) Graph depicting the results from embryonic viability assays. No significant difference in average embryonic viability was observed between control and *wee-1.3* RNAi. (B) Graph depicting the results of brood size assays. A significant reduction in average brood size was observed in males subjected to *wee-1.3* RNAi. Error bars depict standard deviation. P-values were calculated with a paired sample for two means t-test.

3.2 RNA interference of *wee-1.3* causes severe germline defects in males

The reduction in brood size from *wee-1.3* RNAi males could be the result of potential germline defects. To further characterize the effects of *wee-1.3* RNAi on males, whole-mount DAPI staining was performed using *him-8(e1489)* males that were subjected to *wee-1.3* RNAi. While some animals were observed to have a wild-type gonad (Figure 2C), others developed no gonad at all (Figure 2B). To quantify these

findings, animals were scored for one of three phenotypes: individuals with wild-type germ lines, germline defects, such as a small or truncated germ line, and no germ lines (Figure 3). While 100% of the *him-8(e1489)* males subjected to *smd-1* RNAi (n=38) had wild-type germlines, *him-8(e1489)* males subjected to *wee-1.3* RNAi (n=29) were observed to have three different phenotypes. 20.7% of the animals had wild-type germ lines, 3.4% had a germline defect, and 75.9% had no visible germline.

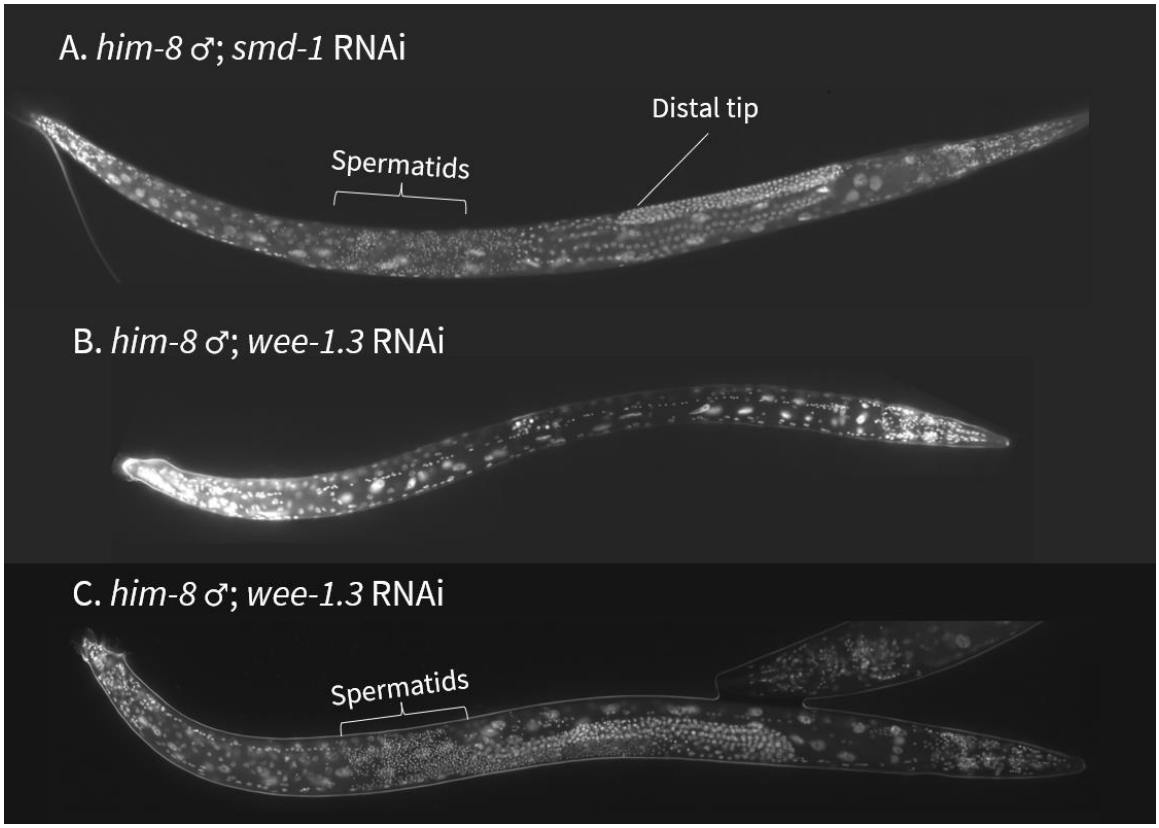

Figure 3.2: Treatment with *wee-1.3* RNAi can prevent males from developing a germ line. Whole-mount DAPI stained *him-8(e1489)* males subjected to either *smd-1* or *wee-1.3* RNAi were imaged at 40x on a Zeiss AxioObserver microscope. Image analysis and stitching was conducted using Fiji software. (A) *him-8(e1489)* male subjected to control *smd-1* RNAi has a wild-type germ line. (B) *him-8(e1489); wee-1.3* RNAi male with no visible germline. (C) *him-8(e1489); wee-1.3* RNAi male with a wild-type germ line.

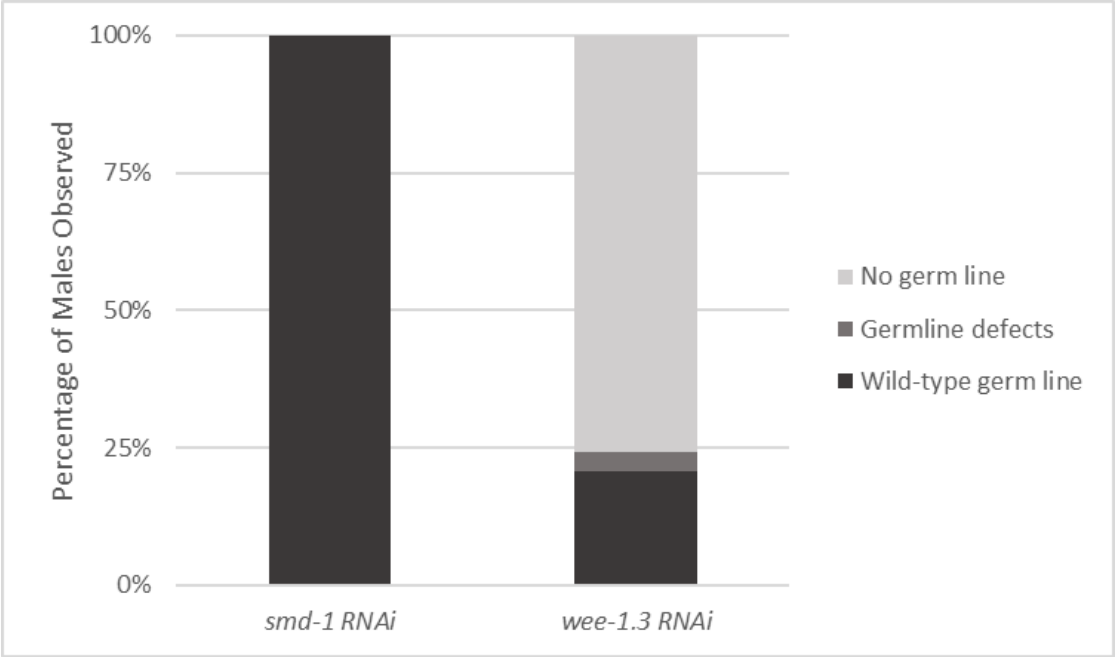

Figure 3.3: Quantification of germline defects observed when *him-8(e1489)* males were exposed to either control (*smd-1* RNAi, n=38) or *wee-1.3* (n=29) RNAi. Black bars represent the percentage of males observed to have wild-type germlines. The dark gray bar represents the percentage of males observed to have germline defects, and the light gray bar represents the percentage of males observed to have no germline.

3.3 WEE-1.3 Localization in the male germ line

In order to determine the pattern of WEE-1.3 expression in male germ lines, males were generated in the *wee-1.3(ana2[GFP::wee-1.3])* and *wee-1.3(ana8[wee-1.3::GFP])* strains by heat shock, which causes X chromosome nondisjunction (Corsi *et al.* 2015). GFP fluorescence in these strains was too faint to be observed via live imaging (data not shown), so gonad dissections and immunostaining against GFP was performed on males from the *wee-1.3(ana8[wee-1.3::GFP])* strain (Figure 4). WEE-1.3 was observed to be expressed throughout the male gonad, with a perinuclear expression pattern in the mitotic zone (Figure 4A). Near the proximal end of the gonad, WEE-1.3 does not appear to be

expressed in spermatids (Figure 4B), although it is expressed throughout the gonad distal to the division zone. This is different from what is observed in hermaphrodites, as WEE-1.3 is expressed in hermaphrodite sperm (Fernando *et al.* 2021).

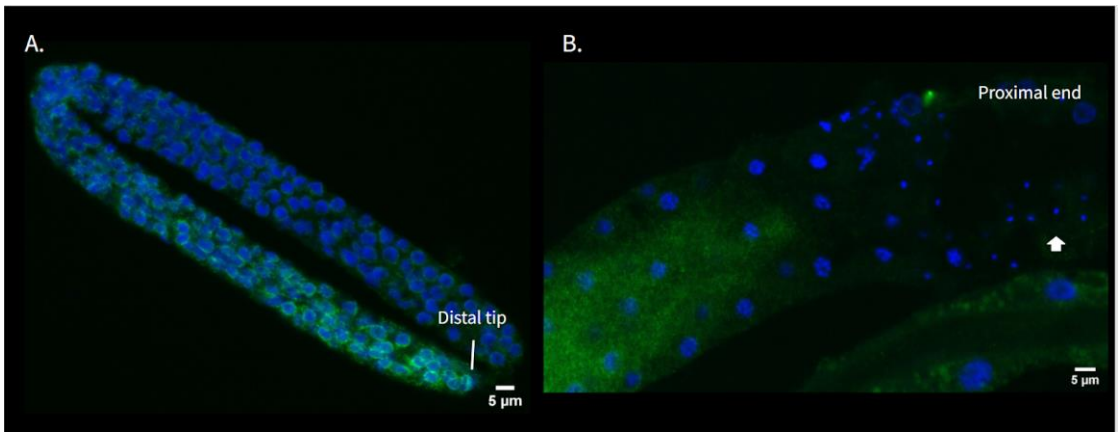

Figure 3.4: Confocal images of *wee-1.3::GFP* dissected male gonads immunostained for GFP captured at 63x with 5 µM scale bars shown. (A) Distal end of a male gonad. (B) Proximal end of a male gonad. White arrowhead indicates spermatids.

3.4 Auxin-inducible Degradation of WEE-1.3

To focus on the germline-specific effects of WEE-1.3 knockdown, we decided to create a system of auxin-inducible degradation for WEE-1.3 in the germ line. We have generated two different *C. elegans* strains where a degron sequence tag has been inserted at the C-terminus of *wee-1.3* using CRISPR/Cas9 genome editing. PCR screening shows the presence of homozygotes with an insertion in *wee-1.3* at the expected size for the *wee-1.3* gene plus the degron sequence (Figure 5). DNA sequencing of purified PCR samples shows that the degron sequence has been inserted at the C-terminus of *wee-1.3* (Figure 6).

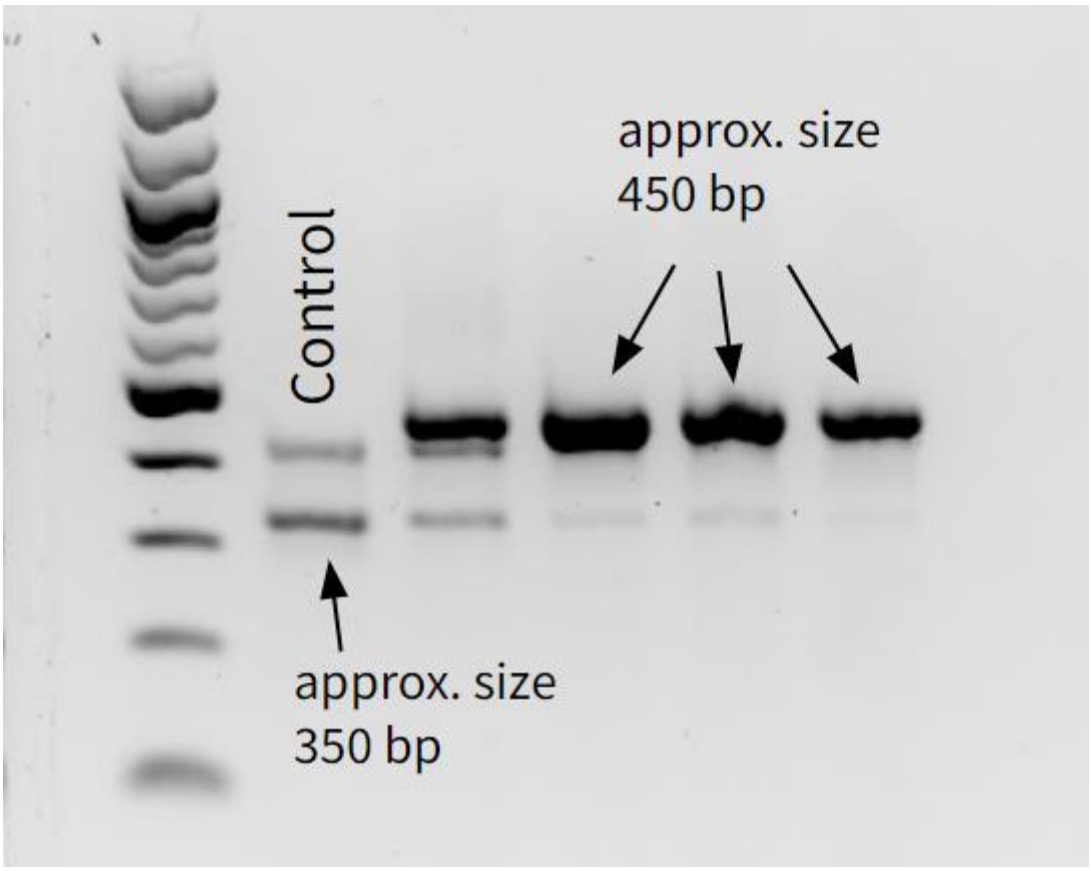

Figure 3.5: DNA gel run with PCR samples from the AJL69 [*wee-1.3(ude32) [wee-1.3::aid]; wrdSi81 (TIR1 germline)*] strain have the expected band size for a degron insertion in *wee-1.3*.

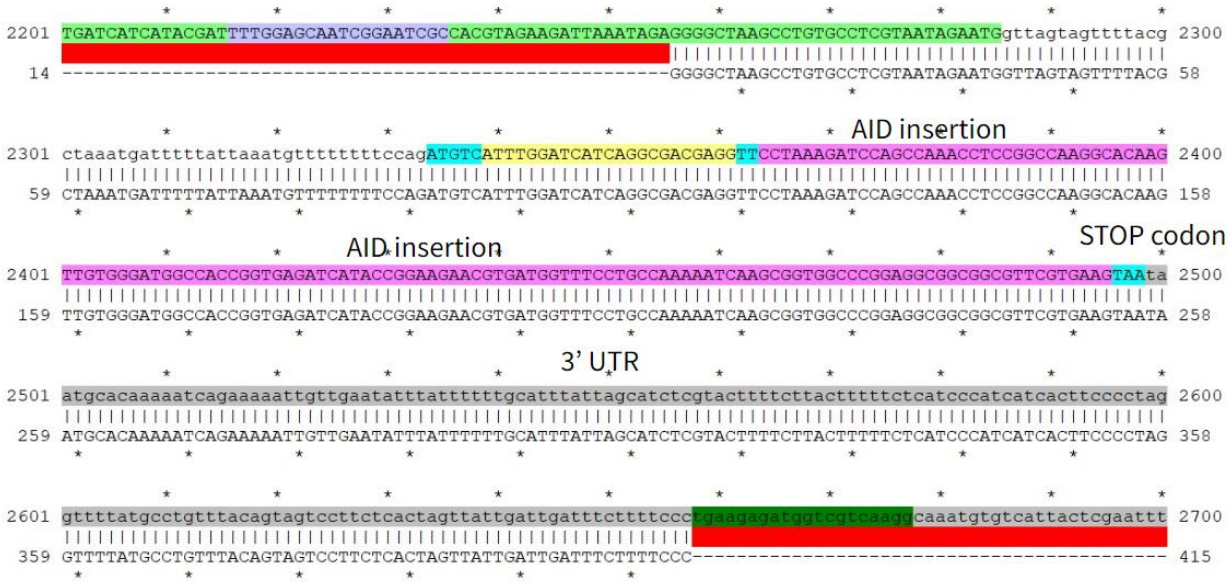

Figure 3.6: Sequencing results for *wee-1.3::aid* CRISPR insertion in the *TIR1 germline* strain. The top sequence is the expected results of the CRISPR insertion, and the bottom sequence is the DNA that was sequenced from the sample. Light green and blue highlighted regions are *wee-1.3* exons, purple highlighting indicates the forward primer used, and dark green highlighting indicates the reverse primer used. The yellow highlighting indicates the *wee-1.3* crRNA used in the CRISPR injection mix (Fernando *et al.* 2021), and the pink highlighted region is the degron sequence.

Chapter 4

DISCUSSION

4.1 RNAi knockdown of *wee-1.3* causes male germline defects

Males subjected to *wee-1.3* RNAi exhibited severe germline defects, including failure to develop a germ line. Other animals appeared to have a truncated or small germ line. This is different from the phenotype observed in hermaphrodites, where *wee-1.3* RNAi causes precocious oocyte maturation (Burrows *et al.* 2006). WEE-1.3 is an essential protein that acts as a regulator during both mitosis and meiosis (Lamitina and L'Hernault, 2002). Complete failure to develop a germline indicates that *wee-1.3* RNAi is causing defects in the mitotic/stem cell niche of the germline. The small, truncated germline phenotype observed could also be due to defects in the mitotic zone of the germline. We also observed that a few males exposed to *wee-1.3* RNAi appear to have wild-type germlines. This could be due to the fact that RNAi is not as highly penetrant in *C. elegans* spermatogenesis (Kamath *et al* 2003). To test the effectiveness of RNAi, we could perform RT-PCR to look for the presence of *wee-1.3* mRNA. As most of the RNAi phenotypes appear to affect mitosis, additional more meiosis-targeted experiments will need to be conducted such as auxin-inducible protein depletion (next section) to observe the roles of WEE-1.3 in meiosis.

4.2 Applications of the *wee-1.3::aid* strain

Unlike RNAi, auxin-inducible degradation can be used to rapidly deplete the target protein in a specific tissue (Zhang *et al.* 2015). To study the effects of WEE-1.3 protein depletion specifically during meiosis of the male germ line, I created a *wee-*

1.3::aid strain that will allow the quick depletion of WEE-1.3 specifically within the *C. elegans* germline. Future experiments will need to confirm that WEE-1.3 is depleted in this strain in the presence of auxin. This will be done by immunostaining against WEE-1.3 after exposure to auxin. In addition, future experiments will include embryonic viability and brood size assays similar to those performed for male *wee-1.3* RNAi to look for evidence of potential male meiotic defects caused by WEE-1.3 depletion in the germline.

4.3 Generating new eGFP-labeled *wee-1.3* strains

Previously, it was published that both the gfp::*wee-1.3* and *wee-1.3::gfp* CRISPR-generated lines were suitable for detecting GFP in hermaphrodites via live imaging (Fernando *et al.* 2021). Unfortunately, I discovered that the WDC2 *(GFP::wee-1.3)* and WDC8 *(wee-1.3::GFP)* strains used for this project no longer express GFP brightly enough to be detected during live imaging. Although I was able to immunostain for GFP to determine the localization of WEE-1.3::GFP at a fixed point in time, this method does not allow for the observation of WEE-1.3 in meiosis in real time. Future experiments include the generation of new, eGFP- and mCherry-labeled *wee-1.3* strains using a method of nested CRISPR/Cas9 (Vicencio *et al.* 2019). Future goals will be to conduct live imaging of males in these strains to better observe expression and localization of WEE-1.3 in spermatogenesis.

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