Spring 5-13-2023

Quantitative and qualitative analysis of mutation in pam-1 of model organism Caenorhabditis elegans.

Jessica Stein

Jessica Stein

Murray State University Honors College

HONORS THESIS

Certificate of Approval

Quantitative and qualitative analysis of mutation in *pam-1* of model organism *Caenorhabditis elegans*.

Jessica Stein

May 2023

Approved to fulfill the
requirements of HON 437

_____
Dr. Chris Trzepacz, Associate Professor
Biology

Approved to fulfill the
Honors Thesis requirement
of the Murray State Honors
Diploma

_____
Dr. Warren Edminster, Executive Director
Honors College

Examination Approval Page

Author: Jessica Stein

Project Title: Quantitative and qualitative analysis of mutation in *pam-1* of model organism *Caenorhabditis elegans*.

Department: Biology

Date of Defense: April 24th, 2023

Approval by Examining Committee:

_____ _____

(Dr. Chris Trzepacz, Advisor) (Date)

_____ _____

(Dr. Gary ZeRuth, Committee Member) (Date)

_____ _____

(Dr. Alexey Arkov, Committee Member) (Date)

Quantitative and qualitative analysis of mutation in *pam-1* of model organism *Caenorhabditis elegans*

Submitted in partial fulfillment

of the requirements

for the Murray State University Honors Diploma

Jessica Stein

May 2023

Abstract

The *pam-1* gene in the model roundworm *Caenorhabditis elegans* governs meiotic exit and establishment of cellular polarity in the single-celled *C. elegans* embryo. Mutation of the *pam-1* gene results in reduced fertility and fecundity in adult *C. elegans* and disrupts the anatomy of the germinal gonad. The aim of this study is to qualitatively and quantitatively define the changes in the germline cells associated with mutations to the *pam-1* gene. Specifically, we examined the stages of germ cell development within the gonads of adult worms, both wild-type and *pam-1* compromised, and identified the changes in the length of the mitotic, transition, and pachytene stages. Our results indicate that the number of pachytene nuclei in mutant strains increases while the number of transition nuclei decreases compared to the numbers in wild-type N2 worms. Changes in the timing of meiotic transitions due to mutation of *pam-1* may disrupt and compromise the maturation of gametes, contributing to the reduced fertility associated with compromised *pam-1* function.

Table of Contents

List of Figures

Introduction

Fertility in humans

In the year of 2002, it was estimated that 186 million women who had ever been married, aged 15-49 years, were reported to be infertile (Rutstein and Shah, 2004). This study was done over several years in developing countries, excluding China, and it's reported that infertility numbers are much higher in less-developed areas than those studied in this publication. Fortunately, their data also saw a decline in infertility over the years, being attributed to increased research on pregnancies and conception, but it is still affecting many individuals worldwide. The term infertility is used to describe many situations regarding the inability to conceive, whether on the side of the male or female, such as sterility or infecundity, the lack of sexual relations, or the inability to conceive a child for a year or more with active efforts regardless of the number of offspring already produced. Plenty of research has been conducted on different forms of assisted reproductive techniques (Inhorn and Patrizio, 2015), but research is still severely lacking on what causes infertility at the cellular level. In 2008, Matzuk and Lamb published research on the highly regulated and coordinated processes of germline development and development of embryos. They found that through studying *Mus musculus*, a mouse model organism for mammals, any disruption to signaling pathways such as sex differentiation, germ cell differentiation, folliculogenesis, and oogenesis, could result in infertility.

Why model organisms are helpful

When learning about how the world around us operates, certain tools can be especially helpful in our quest for knowledge. These tools paint simpler pictures for us so we can more easily understand the complex concepts that we are aiming to interpret. One of these tools is

model organisms. Model organisms are species that are used in laboratory studies to illustrate biological processes and their potential implications for humans. A species is designated as a model organism based on its suitable nature for experimental study, such as a short life cycle, non-specific living requirements, and capacity for genetic manipulation with established techniques. The model organism that we will be focusing on, though, is *C. elegans*.

Why study *C. elegans*?

C. elegans, a microscopic soil nematode, is a prime subject to study in the lab, having a three-day life cycle, small size, and easy laboratory rearing (Riddle et al.). This species naturally self-fertilizes as a hermaphrodite, but can also cross with males of the population, allowing for ease of manipulation of certain genes and/or mutations in that population. Their genome contains approximately 100 million base pairs (Coulson et al., 1986) compared to humans which contain approximately 3 billion base pairs, making it simpler to make genotype to phenotype connections. *C. elegans* has an invariant lineage and is comprised of precisely 959 somatic cells for adult hermaphrodites. The interest of this study, though, is specifically on the gonad of adult *C. elegans*.

Adequate survival of a sexually-reproducing species depends on the development and function of the somatic gonadal tissues and its germline. The germline is responsible for gametogenesis, or production of the gametes (oocytes and sperm). The role of the gonad is to protect the germline, allowing for maturation and proliferation to commence. Both of these tissues need to coordinate their activities in order to be successful for the individual.

Structure/development of the gonad

The reproductive system of adult *C. elegans* hermaphrodites is composed of a mirrored duo of somatic U-shaped gonads that meet at a shared uterus. Each arm of the gonad is connected to the uterus through a spermatheca, the organ holding the sperm (Figure 1, top panel). At the far end of each gonad sits one distal tip cell, or DTC, which caps exposed germ cell nuclei (Fitzgerald and Greenwald, 1995; Hall et al., 1999). The main body of the somatic gonad is comprised of five pairs of gonadal sheath cells and the spermatheca contains 24 somatic cells. Within the spermatheca, immature sessile spermatids mature into mobile amoeboid sperm and wait for the arrival of mature oocytes. The somatic gonad fully encompasses and contacts the germline except for a region between the DTC and the first sheath cell pair (Hall et al., 1999).

The germ cell nuclei that populate the adult hermaphrodite gonad begin their development in a shared syncytial cytoplasm, with the germinal nuclei adjacent to the somatic gonad tissues and circumferentially arranged around a central core, or rachis. These gonad precursor cells develop when the worm is in the first larval stage, or L1. The germline progenitor cells, Z2 and Z3, will develop into the distal tip cells, which, in adults, mark the start of the gonad and provide a stem cell niche for continued mitoses in germ cells. This also serves to regulate proteins to promote germ cell entry into meiosis as they leave this niche (Kimble and Crittenden, 2005). From there, numerous mitotic nuclei form and progress into the transition phase of the nuclei, identifiable by their distinct crescent-shaped look (Figure 2A,B). Here they initiate meiosis. The nuclei continue into the pachytene phase which is identified by its resemblance to a “bowl of spaghetti” (Figure 2C). The gonad bends, making a 180-degree

turn, and the nuclei begin to arrange themselves in a single file, marking the exit of the pachytene stage. The developing oocytes pass through the diplotene stage and arrest semi-permanently in diakinesis in the proximal region of the gonad. The arrested immature oocytes begin to cellularize and increase in volume. Upon reaching the spermatheca and uterus, meiosis is resumed, oocytes complete maturation, and ovulation is able to occur (Hirsch D, et al., 1976). The average brood size for a wild-type worm is ~300 individuals, and greater than 99.9% are viable. This highly reproducible and predictable process is disrupted by mutation. For example, mutation of any of the *C. elegans* orthologs of the conserved Ras/MAPK signaling pathway (Joneson et al., 1996) increases the number of pachytene nuclei in the gonad, resulting in a reduction of fertility. A similar phenotype is also observed in worms harboring mutant *pam-1* alleles.

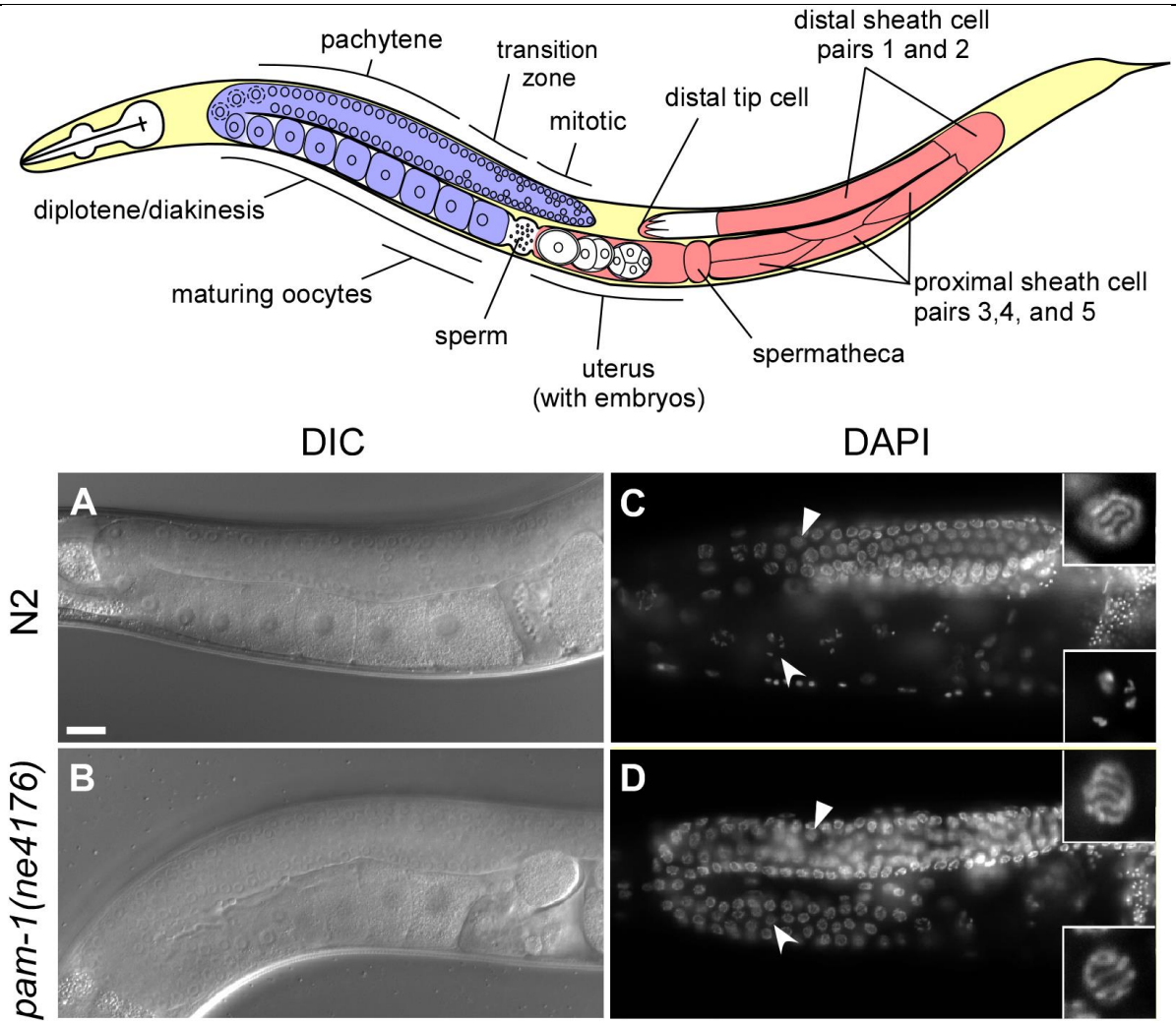

Figure 1. The gonads of *pam-1* hermaphrodites display delays in germ cell development. Top panel, illustration of the *C. elegans* adult hermaphrodite gonad. Germ cells (blue) proliferate and mature within a bilateral somatic gonad (red). Each gonad arm is functionally equivalent, consisting of both germline and somatic components; the split illustration is for simplicity. (**A,B**) DIC images of adult hermaphrodite gonads. The distal gonad is to the top of the U-shaped gonad, the proximal gonad to the bottom. (**C,D**) DAPI-stained fixed adult hermaphrodite gonads similarly oriented. In the gonads of N2 hermaphrodites (panels A and C), the pachytene stage germ cell nuclei of the distal gonad exit pachytene at the bend of the gonad and progress to diakinesis. In *pam-1* gonads (panels B and D), the pachytene zone is expanded and pachytene exit frequently occurs in the proximal gonad. The upper inset images are from similar positions in the distal gonad (arrowhead), the lower inset images from the proximal gonad (arrow). The scale bar represents 20μm.

pam-1, the *C. elegans* ortholog of humans

Puromycin-sensitive aminopeptidase (PSA), PAM-1 in *C. elegans*, is a protein that regulates centrosome positioning in early embryos. It prevents premature movement of the sperm-donated centrosome from the posterior cortex which guarantees proper axis establishment in the growing embryo (Fortin SM, et al., 2010). This protein also has a role in cell cycle progression through centrosome regulation (Lyczak R, et al., 2006). This protein functions by hydrolyzing peptide bonds at the N-terminus of target proteins and although its substrates are not known, it is said to favor small neutral or positively charged amino acids in this terminal position (Brooks et al., 2003).

Mutation of several orthologs of this gene have also been shown to reduce embryonic viability in many different species, including mice, flies, and plants. In 2001, Osada et al. discovered that mice having a PSA deficiency lacked copulatory behavior and had impaired spermatogenesis, resulting in infertility. Shulz found that upon analysis of dPSA, the *Drosophila* ortholog of PSAs, males that only had one wild-type copy exhibited diminished fertility (2001). Sánchez-Morán revealed that Arabidopsis thaliana mutants with diminished or absent PSA activity exhibited reduced fertility and phenotypes characterized by shorter fruits and decreased seed-bearing (2004). This conserved gene is largely supported to be a key factor in reproductive success across many species, including *C. elegans*.

What do mutations to *pam-1* mean for the worm?

Mutations to *pam-1* affect fertility. Two mutant alleles of this gene, one a missense mutation, *pam-1(ne4176)*, and one the result of a deletion, *pam-1(or282)* producing a

frameshift and stop codon, have produced decreased numbers in brood size from 300 to 200 individuals and viability of the embryos from 99.9% to as low as 11.3%

In wild-type N2 hermaphrodite gonads the exit from pachytene and entry into diplotene is completed by the time the germ cell nuclei travel around the gonad bend and enter the proximal gonad (Fig. 1A,B). In contrast, the gonads of *pam-1(ne4176)* animals occasionally (18%) display an expanded region of syncytial germ cell nuclei, past the gonad loop and into the proximal arm (Fig 1C,D). In *pam-1(or282)* animals this defect is over 53% penetrant. The expansion of the syncytial region into the proximal gonad results in fewer oocytes in the *pam-1* gonad (~25% fewer oocytes in ne4176, ~55% in or282 compared to N2, data not shown), and the phenotype is labelled "Pex" (Trzepacz et al., unpublished observations). Because the pachytene stage is immediately adjacent to the gonad flexure and the expanded nuclei are similar in appearance to pachytene stage nuclei, we hypothesize that the mutation of *pam-1* specifically results in an increase in the number of pachytene nuclei in the hermaphrodite gonad of *C. elegans* and developed this study to describe this event qualitatively and quantitatively.

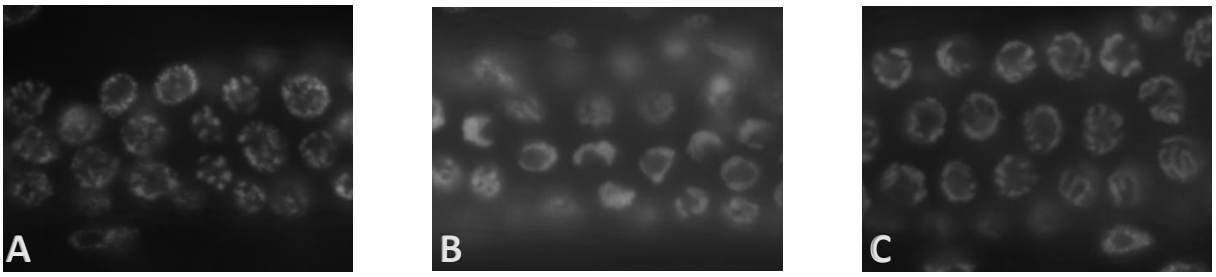

Figure 2. Nuclei imaged from a *C. elegans* gonad. (A) Mitotic nuclei, (B) Transition nuclei, (C) Pachytene nuclei.

Methods

Culturing worms

Following the maintenance and care of *C. elegans* described by Brenner in 1974, placing each strain on NG plates that have been laden with agar media (3 g NaCl, 2.5 Bactopeptone (Difco) and 17 g Bacto-agar (Difco) dissolved in 975 mL distilled water, 1 mL cholesterol in ethanol (5 mg/mL), 1 mM $CaCl_2$, 1 mM $MgSO_4$, and 25 mL potassium phosphate buffer (0.8 M KH_2PO_4, 0.2 M K_2HPO_4, pH 6.0), added in order) and seeded with *E. coli* OP50 to provide a source of food. These require subculturing every ten days or so to ensure the worms have enough food to grow their population's new generations continuously.

Preparing worms for analysis

For simplicity, alphanumeric values will be used to indicate when certain procedures are completed, with the day being indicated by the letter D and the span between them indicated by the accompanied number. On D0 it was made sure that gravid adults were abundant on the plates.

D1 bleach adults

On D1, the gravid adult worms were bleached in order to synchronize the larva to the exact same age. The worms were first transferred from the plate to a test tube with sterile M9 solution (64 g of Sodium Phosphate Dibasic Heptahydrate, 15 g of Potassium Phosphate Monobasic, 2.5 g of Sodium chloride, and 5 g of Ammonium chloride, dissolved in 1 liter of distilled water, autoclaved) and a glass micropipette. After collecting all the worms, a centrifuge was used for 10 seconds at 500 RPM to spin the worms down to a pellet and the supernatant

was removed. Then 10 mL of a 4% sodium hypochlorite solution (131.25 mL household bleach mixed into 868.75 mL distilled water) was added and the worms incubated at room temperature, dissolving, until the test tube contained only embryos and minimal debris. The liberated embryos were spun down and as much of the supernatant and debris possible was vacuumed off without disturbing the pellet of embryos. The embryos were washed with M9 solution, spun down, and the supernatant removed for a total of three washes to remove as much of the bleach as possible. After adding 7 mL of M9 solution to the test tube, the liberated embryos were left to grow in solution on an automated rotator overnight.

D2 plate starved L1's

Having left the embryos overnight on the rotator, they had grown to the L1 stage and were arrested in development there due to the lack of food. The larvae were transferred to fresh culture plates, with each plate having around 40 individuals to one drop of OP50 *E. coli* for food.

D5 fix adults

With the larvae plated on D2, they were left for three days to grow into gravid adults. Gravid adults were transferred to a microcentrifuge tube with M9 solution and a glass micropipette, spun down in a centrifuge, and the supernatant was vacuumed off of the pellet of worms. 150 µL of -20°C methanol was added to the tube and the tube incubated for 15 minutes at -20° C. After resting in the methanol, the solution was spun down again and the supernatant was vacuumed off. The worms were washed with M9, spun down, and supernatant vacuumed off to remove as much methanol as possible. This process was repeated two more times. 1.0 mL of sterile M9 supplemented with 1µL of 1 mg/mL DAPI (4',6-diamidino-2-phenylindole

dihydrochloride) was added to the worm pellet, and this solution was allowed to incubate for 10 minutes to adequately fluorescently stain the nuclei of the worms. The solution was spun down in the centrifuge, vacuumed, and washed three more times to remove excess DAPI.

Microscopy

An agarose gel pad was made using 130 microliters of warmed 2% agarose solution and two glass microscope slides to cool and set the pad. Approximately 15 worms were added to the pad and a cover slip was placed on top.

Worm gonads were illuminated with UV light at 358 nm and examined with a 461 nm DAPI filter. All observations were made at 1000x magnification.

Syncytial nuclei were counted at the gonad periphery (Figure 3) of each phase in the germline: mitotic, transition, and pachytene (Figure 2). Exit from the pachytene stage in N2 gonads occurs before entry into the proximal gonad. An arbitrary border perpendicular to the proximal gonad arm, passing through the apex of the inner curvature of the gonad was used and gonads displaying pachytene stage germ cell nuclei past this proximal border were scored as delayed and having a Pex phenotype (Althoff et al., 2014).

Statistical significance between mean nucleic counts of different strains and stages was determined through two-tailed independent t-tests assuming unequal variances.

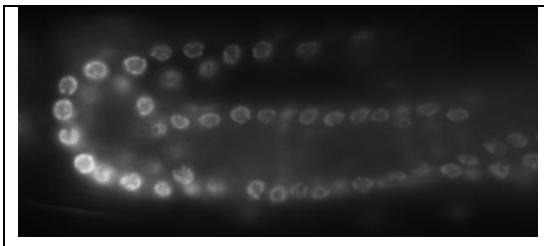

Figure 3. Nuclei imaged at the gonad periphery

Results

The nuclei of 140 separate gonads were observed and assessed through each of the three phases, mitotic, transition, and pachytene. Gonads numbered 54 in the N2 strain, with 0 exhibiting Pex, 46 in the *pam-1(ne4176)* strain, with 22 exhibiting Pex, and 40 in the *pam-1(or282)* strain, with 20 exhibiting Pex. There was statistically significant difference between the total number of gonad nuclei in N2 and *pam-1(ne4176)*, but not between N2 and *pam-1(or282)* or N2 and either mutant strain displaying the Pex phenotype (Figure 4, 5). Both mutant strains exhibiting the Pex phenotype had a larger amount of pachytene nuclei present. We found that there was a statistically significant difference between N2 (M=22.111, SD=3.606) and *pam-1(ne4176)* exhibiting a Pex phenotype (M=26.087, SD=2.778) on the number of pachytene nuclei, $t(53) = -5.24$, $p < .05$, as well as between N2 (M=22.111, SD=3.606) and *pam-1(or282)* exhibiting a Pex phenotype (M=26.350, SD=2.601) on the number of pachytene nuclei, $t(47) = -5.57$, $p < .05$ (Figure 5). Both mutant strains exhibiting the Pex phenotype had a smaller amount of transition nuclei present. There was also a statistically significant difference between N2 (M=16.778, SD=2.963) and *pam-1(ne4176)* exhibiting a Pex phenotype (M=13.652, SD=1.824) on the number of transition nuclei, $t(65) = 5.68$, $p < .05$, as well as between N2

(*M*=16.778, *SD*=2.963) and *pam-1(or282)* exhibiting a Pex phenotype (*M*=11.900, *SD*=2.614) on the number of pachytene nuclei, t(38) = 6.87, p < .05 (Figure 5). Finally, there was a statistically significant difference between N2 (*M*=20.944, *SD*=3.206) and *pam-1(ne4176)* exhibiting a Pex phenotype (*M*=17.913, *SD*=2.087) on the number of mitotic nuclei, t(62) = 4.92, p < .05, but no significance between N2 and *pam-1(or282)* on mitotic nuclei. A t-test indicated that there was no significant difference between *pam-1(ne4176)* (*M*=26.09, *SD*=2.778) and *pam-1(or282)* (*M*=26.35, *SD*=2.601) on the number of pachytene nuclei from a Pex phenotype, t(41) = -0.32, *p* = 0.75, but there was a statistically significant difference between their mitotic stage, t(36) = -3.69, *p* < .05, *pam-1(or282)* having a larger average count (*M*=20.65 vs. *M*=17.91), and transition stage, t(33) = 2.513, *p* = < .05, *pam-1(ne4176)* having a larger average count (*M*=13.65 vs. *M*=11.90) (Figure 5).

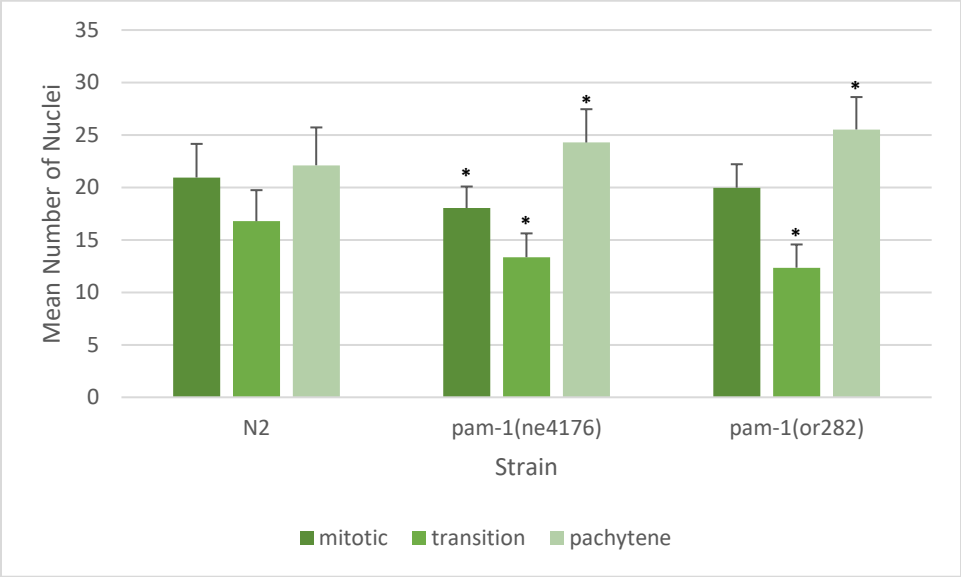

Figure 4. Average nuclei counted in gonad periphery between mitotic, transition, and pachytene stages in three strains of *C. elegans*, N2 (n=54), *pam-1(ne4176)* (n=46), and *pam-1(or282)* (n=40) with error bars representing the standard deviation of each group. * Indicates statistical significance between the mutant strain and N2 worms

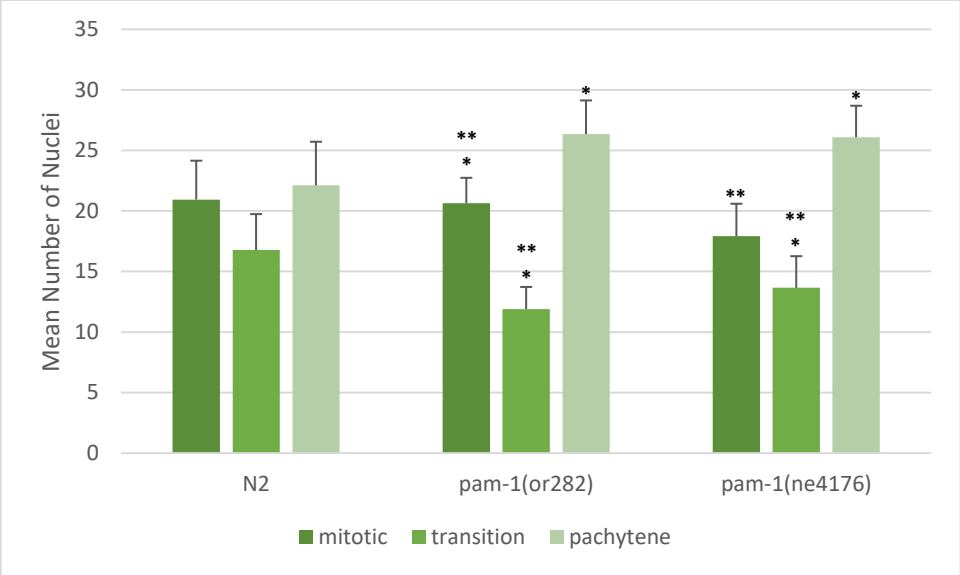

Figure 5. Comparison of average nuclei counted in gonad periphery between mitotic, transition, and pachytene stages in N2 and Pex phenotype of two mutant strains of *C. elegans*, *pam-1(ne4176)* (n=23) and *pam-1(or282)* (n=20) with error bars representing the standard deviation of each group. **Indicates statistical significance between the two mutant strains exhibiting Pex

Discussion

The aim of this study was to determine if a mutation to *pam-1* resulted in an increased count of pachytene nuclei in the hermaphrodite gonad of *C. elegans*. Our data shows that compared with N2 worms, the *pam-1(ne4176)* mutant had a smaller mean count of mitotic nuclei, a smaller mean count of transition nuclei, and a larger mean count of pachytene nuclei. Comparing N2 to the *pam-1(or282)* mutant revealed no statistically significant difference in mitotic nuclei count, a smaller mean count of transition nuclei in the mutant, and a larger mean count of pachytene nuclei in the mutant. Comparing the total number of nuclei present in N2 and each mutant strain displaying the Pex phenotype, we determined that there was no statistical significance to the difference in numbers, suggesting that the expansion of the pachytene stage is mitigated by a change in some other factor. This analysis supports our hypothesis that a relationship exists between mutation to *pam-1* and an increased number of

pachytene nuclei. A relationship also seems to be present between mutation to *pam-1* and a decreased number of transition nuclei. As this result was not hypothesized beforehand, a further look into this relationship is warranted. It's possible that *pam-1* could be in close proximity to a regulator of the transition stage, such as any number of the proteins involved in the RAS/MAP kinase pathway, therefore disrupting the sequence of development and DNA synthesis in the gonad. These pathways and processes are highly regulated, so when mutations occur to one aspect of the overall system, like the RAS/MAP kinase pathway, ectopic expression or complete loss of function can result and produce a multitude of phenotypes (Joneson et al., 1996). Something else that is of interest to us is the result that the total nuclei count of the mutant strains expressing the Pex phenotype are not significantly different than the N2 worms. Although we do see an increase in the number of pachytene nuclei, there is a decrease in the number of transition nuclei to offset that, resulting in our similar total counts. Perhaps the extension of the pachytene stage into the proximal gonad is due to increased space in between each nucleus as well as a possible decreased size of the gonad, future data collection on these characteristics should be completed in order to determine this relationship.

Moving forward with these results, I think it would be beneficial to not only explore the relationship between the mutation to *pam-1* and the count of transition nuclei decreasing, but also to explore different strains of *C. elegans* that result in an expanded syncytial (Pex) gonad. Since there is not a large quantity of research on *pam-1* and its mutant strains, looking at other mutant strains that produce the same phenotype might reveal some of PAM-1's substrates that are currently unknown (Brooks et al., 2003). This research, once more data has been collected and the function of *pam-1* fully realized, could have some major implications for the human

race. We still struggle with infertility as research and people in our own lives testify to daily, and the majority of the solutions that have been proposed to combat this treat the symptoms of infertility, not the original problem (Inhorn and Patrizio, 2015). While this is still extremely important, there are still unanswered questions as to why this is happening in the first place and what is causing it on the cellular level. Looking at the conservation of PAM orthologs across various species gives the potential for an answer to this problem (Shulz et al., 2001, Sánchez-Morán et al., 2004, Osada et al. 2001).

Acknowledgements

I would like to personally thank Dr. Chris Trzepacz for educating and guiding me through the process of cultivating, observing, and analyzing the worms over the last three years. It has been an amazing learning experience sandwiched between some of the best jam sessions and most interesting conversations I have had at this university. I'm definitely going to miss it.

References

Althoff MJ, Flick K, Trzepacz C. Collaboration within the M1 Aminopeptidase Family Promotes Reproductive Success in Caenorhabditis elegans. Development genes and evolution. 224:137-146; 2014.

Brenner S. The genetics of Caenorhabditis elegans. Genetics. 77(1):71-94; 1974.

Brooks DR, Hooper NM, Isaac RE. The Caenorhabditis elegans Orthologue of Mammalian Puromycin-Sensitive Aminopeptidase has Roles in Embryogenesis and Reproduction. Journal of Biological Chemistry. 278(44):42795-84201; 2003.

Coulson A, Sulston J, Brenner S, Karn J. Toward a Physical Map of the Genome of the Nematode Caenorhabditis elegans. Proceedings of the National Academy of Sciences. 83(20):7821-7825; 1986.

Fitzgerald K, Greenwald I. Interchangeability of Caenorhabditis elegans DSL Proteins and Intrinsic Signaling Activity of their Extracellular Domains in vivo. Development. 121(12):4275-4282; 1995.

Fortin SM, Marshall SL, Jaeger EC, Greene PE, Brady LK, Isaac RE, Schrandt JC, Brooks DR, Lyczak R. The PAM-1 aminopeptidase regulates centrosome positioning to ensure anterior–posterior axis specification in one-cell C. elegans embryos. Developmental biology. 344(2):992-1000; 2010.

Hall DH, Winfrey VP, Blaeuer G, Hoffman LH, Furuta T, Rose KL, Hobert O, Greenstein D. Ultrastructural Features of the Adult Hermaphrodite Gonad of Caenorhabditis elegans: Relations Between the Germ Line and Soma. Developmental biology. 212(1):101-123; 1999.

Hirsh D, Oppenheim D, Klass M. Development of the reproductive system of Caenorhabditis elegans. Developmental biology. 49(1):200-19; 1976.

Hubbard EJA, Greenstein D. The Caenorhabditis elegans Gonad: A Test Tube for Cell and Developmental Biology. Developmental Dynamics: Reviews; 2000.

Inhorn MC, Patrizio P. Infertility Around the Globe: New Thinking on Gender, Reproductive Technologies and Global Movements in the 21st century, Human Reproduction Update, Volume 21, Issue 4, Pages 411–426; 2015.

Joneson T, White MA, Wigler MH, Bar-Sagi D. Stimulation of Membrane Ruffling and MAP Kinase Activation by Distinct Effectors of RAS. Science. 271(5250):810-812; 1996.

Kimble J, Crittenden SL. Germline proliferation and its control. Howard Hughes Medical Institute, Department of Biochemistry, University of Wisconsin, Madison, WI; WormBook: The C. elegans Research Community; 2005.

Lyczak R, Zweier L, Group T, Murrow MA, Snyder C, Kulovitz L, Beatty A, Smith K, Bowerman B. The puromycin-sensitive aminopeptidase PAM-1 is required for meiotic exit and anteroposterior polarity in the one-cell Caenorhabditis elegans embryo. The Company of Biologists: Development; 2006.

Matzuk MM, Lamb DJ. The Biology of Infertility: Research Advances and Clinical Challenges. Nature Medicine, 14(11):1197-1213; 2008.

Osada T, Watanabe G, Kondo S, Toyoda M, Sakaki Y, Takeuchi T. Male Reproductive Defects Caused by Puromycin-sensitive Aminopeptidase Deficiency in Mice. Molecular Endocrinology. 15(6):960-71; 2001.

Riddle DL, Blumenthal T, Meyer BJ, et al., editors. C. elegans II. 2nd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1997. Section I, The Biological Model. Available from: https://www.ncbi.nlm.nih.gov/books/NBK20086/

Rutstein SO, Shah IH. Infecundity, Infertility, and Childlessness in Developing Countries. DHS Comparative Reports: 9; 2004.

Sánchez-Morán E, Jones GH, Franklin FC, Santos JL. A Puromycin-sensitive Aminopeptidase is Essential for Meiosis in Arabidopsis Thaliana. The Plant Cell. 16(11):2895-2909; 2004.

Schulz C, Perezgasga L, Fuller MT. Genetic Analysis of dPsa, the Drosophila Orthologue of Puromycin-sensitive Aminopeptidase, Suggests Redundancy of Aminopeptidases. Development Genes & Evolution. 211(12); 2001.