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The PAM-1 aminopeptidase protects against neurodegeneration in Caenorhabditis elegans

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Murray State University Honors College

HONORS THESIS

Certificate of Approval

The PAM-1 aminopeptidase protects against neurodegeneration in *Caenorhabditis elegans*

Caleb Coil

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The PAM-1 aminopeptidase protects against neurodegeneration in *Caenorhabditis elegans*

Submitted in partial fulfillment
of the requirements
for the Murray State University Honors Diploma

Caleb Coil

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A special thanks to Dr. Chris Trzepacz for his direction and guidance during my tenure in the Worm Lab. Thank you to the Murray State University Department of Biological Sciences for making this project possible.

Abstract

The deteriorating effects of neurodegenerative conditions seem inescapable for many as they age. The experimental exploration of the molecular and cellular mechanisms responsible for neurodegeneration is aided using animal models. For instance, genetic screens have identified the puromycin-sensitive aminopeptidase (PSA) as a novel effector of neurodegeneration, as mutations in PSA in fruit flies result in perturbations of neuron anatomy and an accelerated onset of neuron death. We have tested the hypothesis that PAM-1, the *Caenorhabditis elegans* ortholog of PSA, plays a similar role in governing neurodegeneration in nematodes. Genetic strains were created that express the green fluorescent protein (GFP) in the six mechanosensory neurons of wild-type and *pam-1* mutant *C. elegans*. Because *C. elegans* is transparent, each of these GFP-labeled strains produce animals that brilliantly illuminate the invariant position, anatomy, and neurodegeneration of a specific subset of neurons. The degeneration can be seen as a natural consequence of aging in the wild-type worms. In the *pam-1* mutant worms, however, a higher prevalence and earlier appearance of similar defects is witnessed. Additionally, it seems that the mutated worms have a shorter lifespan than their wild-type counterparts. The *pam-1* mutation, its effects, and the research done with *C. elegans* sheds light on neurodegeneration in a manner impossible for human subjects.

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Introduction

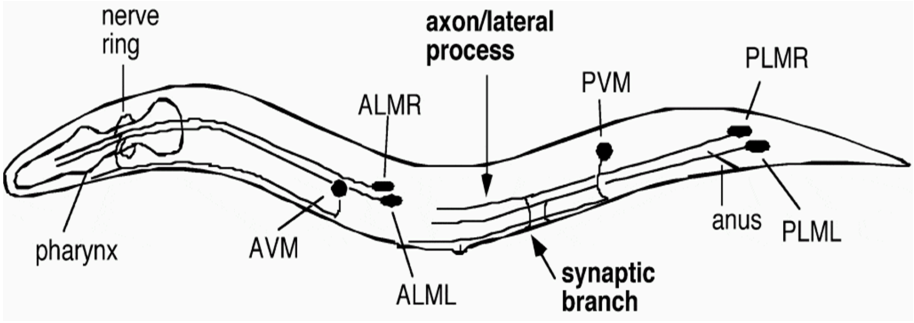
Caenorhabditis elegans as a model organism

Discovered well over a century ago, the *Caenorhabditis elegans* has had a profound impact on scientific research and knowledge ever since. First termed as the *Rhabditis elegans* by Emile Maupas, the *C. elegans* has become a renowned model organism (Nigon et al.). The nematode, measuring a mere millimeter in length when full-grown, is nothing more than a soil species. It thrives on bacteria, but, unlike many of its nematode cousins, it is in no way pathogenic itself. *C. elegans* research can be traced to the start of the 20th century, and the most noted beginnings of this nematode's journey to scientific fame were made in the 1960s by Sydney Brenner (Nigon et al.). Early studies were interested in the survival-driven "dauer" phase of worm growth. The nematode quickly became a model for reproductive and developmental studies as well, and this really has never changed (Nigon et al.) Most importantly, the worm, as it is simply called, consists of 959 somatic cells (or 1031 in the males), and is unique among metazoan models in that the fates of these cells are invariant in lineage (Bao et al.). Combined with the fact that the worm and its organs are largely transparent, the *C. elegans* is an obvious choice for the real-time, *in vivo* study of conserved metazoan organ, tissue, and cellular processes including development, reproduction, and neurology.

C. elegans neuroanatomy

As technology and understanding of genetic processes have developed, scientific research endeavors of all kinds have grown. Nonetheless, the invaluable *C. elegans* has continued to be a prime species for work in the lab. A key discovery along the way has been the mapping of the

worm's neurons. The *C. elegans* is now known to contain 302 neurons that are grouped into many ganglia (Hobert). Hobert continues that there are anterior and posterior ganglia found in the head and tail of the organism, respectively, as well as more ganglia found in the dorsal and ventral subregions. The lineages of these neurons, which are typically non-clonal, demonstrate the complexity found in such a small organism (Hobert). The nature of these neurons has prompted them to be classified based on positioning and connections within the worm (Hobert). While formulating nomenclature of the few hundred neurons may have been difficult, viewing them can be quite simple. The *C. elegans* has six mechanosensory neurons (Cohen et al.). Three are located in the anterior of the animal (AVM, ALML, ALMR; see **Figure 1**, upper panel), and three are located in the posterior of the animal (PVM, PLML, PLMR). These namings are examples of the "axodendritic projection" patterns that Hobert refers to in his book. For instance, AVM denotes the anterior ventral microtubule cell. In the wild-type strain of worms we have used, known as CZ10175, the *zdIs5* transgene expresses a green fluorescent protein (GFP) under the *mec-4* promoter to drive expression of GFP in just these six neuronal cells. One exceptional characteristic of the worm is its invariant body plan. The fate of all cells, including neurons, are known and do not change, save major mutations. Because of this, the *zdIs5* transgene will repeatably induce GFP expression in the same manner in the same exact six mechanosensory neurons.



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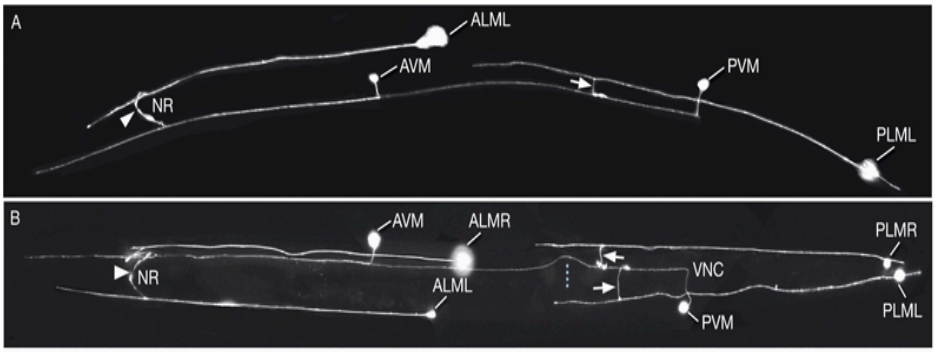

https://www.wormatlas.org/hermaphrodite/nervous/Neuroframeset.html

Figure 1. The mechanosensory neurons of *C. elegans*. Both the top and bottom panels display the six cell bodies and their processes in relation to the body layout of the worm.

Understanding of this has been used to explore the connectome and how neuronal signaling works inside the worm. Furthermore, there are many parallels between the *C. elegans* and the human body. Because of this, neurological disorders have become a targeted area of research for the worm. The worm is well-suited for studying the impact of mutations on cellular form and function. Understanding why signal breakdown occurs, how aggregated proteins occur, and what genes are involved are within the realm of *C. elegans* research.

Puromycin Sensitive Aminopeptidase

The puromycin sensitive aminopeptidase (PSA) is a conserved enzyme whose homologs are found in many different organisms. During initial testing, it was determined that the aminopeptidase was inhibited by the presence of the antibiotic puromycin, resulting in the name (Madabushi et al.).

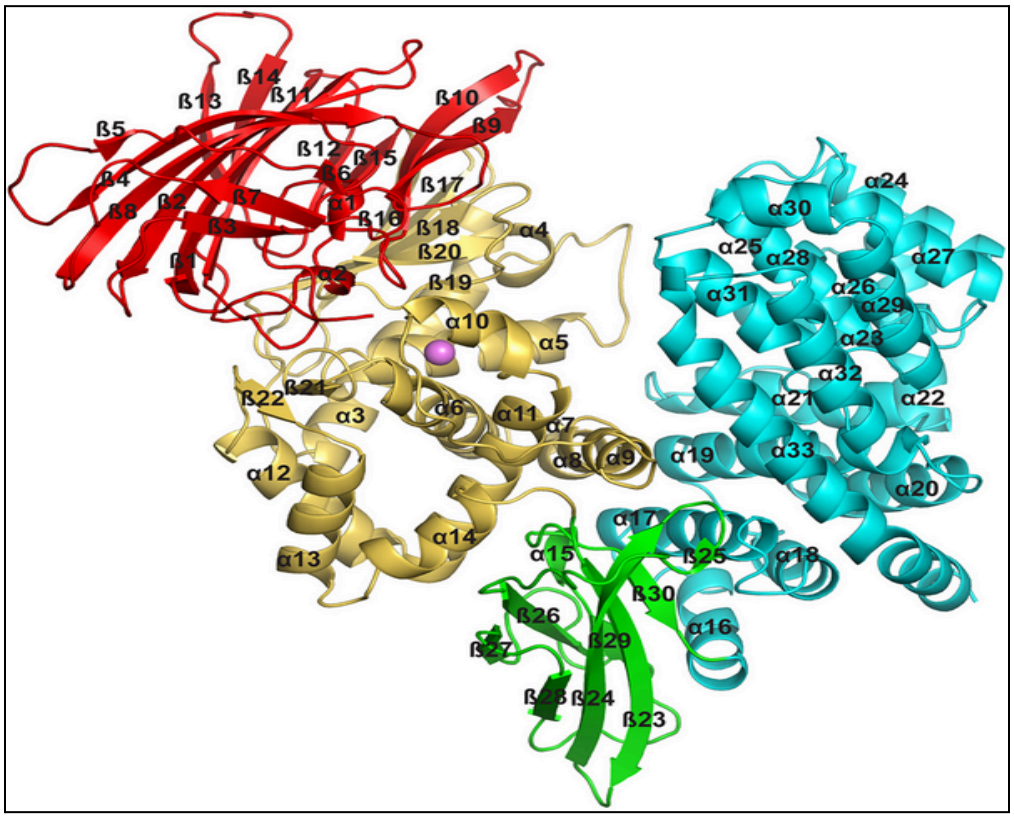

Figure 2. Puromycin Sensitive Aminopeptidase. The four domains are shown, in addition to the pink sphere that represents the zinc ion cofactor. The catalytic groove can also be seen.

PSA is considered a zinc metallopeptidase in the M1 family. It appears in a V-shape, consisting of four domains with a 15-angstrom-wide groove (Madabushi et al.). Madabushi et al.

further state that the zinc cofactor is stabilized via interactions with two histidine residues and a glutamic acid residue within the $HEXXHX_{18}E$ active site sequence. A molecule of water additionally coordinates the zinc ion and acts as a nucleophile in catalysis. When the glutamic acid (in its base form, glutamate) takes a proton from the water, a nucleophilic reaction between the enzyme and a protein substrate can occur, enabling enzymatic functionality (Madabushi et al.). Madabushi et al. explain that, in another amino acid sequence of the PSA (the "GAMEN" sequence), a glutamate residue is thought to be responsible for binding substrates at their amino terminus. Through these processes, PSAs serve to degrade and destroy toxic substances within the body--typically, sequences of amino acids such as glutamine chains (Bhutani et al.). Degradation of these residues, often 30 or more residues in length, is important in warding off disease in the brain of an organism, which has far-reaching effects on many systems of the body. NPEPPS, the version of the PSA enzyme found in many human tissues, plays a role in cell polarity, cell cycle control, and, again, the degradation of some aggregates released by the proteosome (Madabushi et al.). Inhibition of the PSA in muscle additionally leads to increases in the problematic huntingtin exon, which is known to create functional declines of the organism (Madabushi et al.).

Tau

Tau is a protein that associates with microtubules, such as those comprising the mechanosensory neurons found in *C. elegans*. Its accumulation has been associated with major illnesses like Alzheimer's Disease (Sengupta et al.). More specifically, groups of proteins slowly accumulate within nervous tissues, such as the brain, as an organism ages. Some of the major

culprits are beta amyloid plaques and tau proteins (Guillozet et al.). As time progresses, these aggregates can form neurofibrillary tangles (NFTs). At the more severe stages, the NFTs will begin to cause the well-known cognitive deficits associated with common neurodegenerative diseases (Guillozet et al.). However, there are mechanisms in place that help fight these types of neuronal decay in some organisms. The *Drosophila melanogaster* ortholog of PSA, dPSA, has been implicated in preventing such neurodegeneration (Karsten et al.). The fruit fly's PSA has been shown to be crucial in prevention of tau protein aggregates, which can preemptively avoid development of NFTs. During testing, mutations in the fly's dPSA were found to promote neurodegeneration (Karsten et al.). Hyperphosphorylation of the tau proteins is believed to be the driving cause behind this neuronal decay (Kudo et al.). The link between dPSA and PSAs in general was further strengthened as more research was done; when researchers overexpressed dPSA in the flies, the neurodegeneration that had been induced by tau proteins was actually reversed (Karsten et al.). This is a powerful piece of information: not only has dPSA been found as a safeguard to neurodegeneration, but it can help restore the effects of tau-induced neuronal deficits. The proteolytic nature of PSAs is, therefore, a necessary part of organismal longevity, but also a potential weakness if the PSA begins to fail or mutate.

PAM-1

PAM-1 is the *C. elegans* ortholog of PSA (Althoff et al.). PAM-1 also falls into the M1 family of metalloaminopeptidases. According to the work done by Brooks et al., the genetic code of *pam-1* encodes the zinc cofactor site and GAMEN sequence found in other M1 aminopeptidases that is required for enzymatic activity. Again, these are key structures within the

aminopeptidase that largely contribute to its role in proteolysis; the similarity in encoded sequences and domains leads to comparable functions between PSA homologs. Like its PSA cousins, PAM-1 plays a role in reproductive success and the viability of embryos (Althoff et al.). Mutations to the *pam-1* gene have resulted in disruption and delay of the meiotic cycle in the nematodes, leading to delays of maturation (Althoff et al.). Not only this, but *pam-1* embryos fail to polarize their anteroposterior axis (Lyczak et al.). Establishment of polarity is an early and fundamental process of worm development, and its disruption can lead to development delays and lethality. Thus, gonadal impairments and decreases in fecundity are well-linked to the mutation of PAM-1.

So the question arises, "Because PAM-1 of *C. elegans* fulfills similar responsibilities as PSAs in other organisms, could mutation of *pam-1*increase neurodegeneration?". We have sought to answer this by comparing strains of worms that have a significant genetic mutation to the *pam-1* gene with strains that are "wild type" and do not have the mutation. The goal is to better understand how the mutation impacts the neuronal pathways of the worms. In this report, we have utilized a transgenic strain that fluorescently labels specific neurons to assess the role PAM-1 plays in neurodegenerative protection. We have hypothesized that mutations that compromise PAM-1 function will increase both the rate and prevalence of degeneration in the worm's neurons.

We have crossed this GFP transgene into a mutant strain of worms with a *pam-1(ne4176)* background, producing the transgenic strain TRZ20. These worms have clearly illuminated mechanosensory neurons, in addition to a mutated *pam-1* gene in the background (**Figure 1**). We have utilized these two strains to perform a longitudinal analysis of *pam-1*-dependent neurodegeneration *in vivo*. Age synchronized, adult hermaphrodite worms of both the wild-type

and mutant genotypes were tracked starting from their first day as an adult (Day 1) for up to 20 days. They were examined at regular intervals (several times each week), and any visual changes in the processes and cell bodies of the neurons were documented.

While aging is correlated with degradation of neurons in any strain of *C. elegans*--or any animal for that matter--our hypothesis is that mutating the gene responsible for PAM-1 expression will lead to either more severe neuronal decay, or at least a faster decline in neuron health. In order to test this, we first had to understand the increasing entropic appearance of neurons in wild-type worms as they aged; we then needed to perform the same longitudinal study with the mutants. The information has been documented and compiled to determine what changes in neurodegeneration, if any, are seen when PAM-1 is compromised.

Methods

C. elegans culture and maintenance

The *C. elegans* strains were kept in a climate-controlled environment set at 20°C. The nematodes were cultured on a three-mL pad of Nematode Growth Media (0.25% tryptone, 0.3% NaCl (50mM), 1.5% agar, 1mM $CaCl_2$, 1mM $MgSO_4$, 20 mM KH_2PO_4, 5 mM K_2HPO_4, 5 mg/mL cholesterol).

The media was created in one- or two-liter batches, with deionized water being added to attain media pH of 6.0. It was then autoclaved for sterilization. For most plating, a 35 mm plate was used, though some 60 mm plates were used for larger populations of worms. Regular feeding of *E. coli* bacterial strain *OP50* (50 µL per plate) was used to sustain the worms throughout their lifespans for the research project.

CZ10175 is a wild-type strain of the *C. elegans* that carries *zdIs5*, a transgene that expresses the green fluorescent protein (GFP) specifically in the six mechanosensory neurons. The *pam-1(ne4176)* strain has a hypomorphic allele of *pam-1*. The *ne4176* mutation encodes an S242R substitution, which replaces a conserved amino acid in the catalytic domain of the aminopeptidase. CZ10175 was genetically crossed with *pam-1(ne4176)* to create TRZ20. Both CZ10175 and TRZ20 robustly express GFP in the six mechanosensory neurons, and TRZ20 worms contain the mutated PAM-1 aminopeptidase.

To create age synchronized populations of worms, several plates of gravid adult hermaphrodites were washed into a 15 mL centrifuge tube using sterile M9 (20 mM KH_2PO_4, 40 mM Na_2HPO_4, 85 mM NaCl, 1 mM $MgSO_4$). Following this, the worms were subsequently spun down, bleached with a hypochlorite solution (0.525% NaHCl, 250 mM KOH) until lysis, and rinsed with M9. Any potential contaminants were now removed. The precipitate, mostly consisting of now-sterile embryos, was placed in seven-mL of sterile M9 and set to rock on a rotisserie shaker.

Within 72 hours, the eggs were hatched and proceeded through the four successive larval stages. On the third day, the *C. elegans* became “Day 1 adults,” and that marked the beginning of the analysis being performed for this study. In this manner of preparing a study population of worms, longitudinal research was able to be conducted using an identical population of time-synchronized worms.

Scoring neurodegeneration

To effectively identify the degeneration of neurons in the nematodes, the following procedures were used, first with the CZ10175 (*zdIs5)* strain of worms, and then with the TRZ20 (*pam-1(ne4176);zdIs5*) strain.

Using a micropipette, a 150-µL pad of two percent agarose (in M9) was placed upon a microscope slide. A 10-µL drop of 1.0 mM tetramisole was then pipetted atop the pad. From the stock of wild-type worms, several *C. elegans* were transferred into the tetramisole, which effectively subdued their movements. Depending on the amount of worms still alive on a given day, an average of 11 worms were studied for each day of adult life (ranging from four worms up to 25). A thin, glass cover slip was eased onto the pad of worms, and the slide was affixed on the microscope stage. The microscope used for the specimen analysis was an Olympus BX51 microscope fitted with the appropriate Nomarski filters. Imaging of the worms was done with the affixed camera--a Q-Color3 CCD camera (Olympus), and Q Capture Pro software (Olympus). The worms were studied under 600x magnification, utilizing oil immersion for clarity, and a UV light to fluoresce the GFP-bearing neurons. Each worm was assessed for the absence or presence of blebbing and/or branching within the neurons. A bleb was characterized as an abnormal, round protrusion within the axon of the nerve cell. A branch was characterized as an atypical long, narrow spur from either a nerve cell body or axon. Data was collected, and pictures of some specimens were captured using the camera. This process was continued at regular intervals through the lifespan of the CZ10175 worms, and this was repeated several times with different generations of worms. Under the microscope, axons, cell bodies, commissures, and other processes were distinguishable. The pictures were used to take regular notes of how the neurons

of the wild-type worms changed over time. The same process--including time synchronization, plating, microscopy, and data collection--was then applied to the strain of *pam-1* mutant worms.

Results

The longitudinal study of the wild type strain CZ10175 and the mutant strain TRZ20 allowed for congruent study of neurodegeneration over the worms' lifespans. Images using the Olympus camera and software depict the progression of neuronal degeneration in both the wild-type and mutant worms. It should be understood that neurons appeared bright green when viewed under the ultraviolet light of the microscope. The camera could capture this in different hues and contrasts, and not all photos will portray the GFP as effectively as in-person. However, the neuronal defects that were present can clearly be seen in the representative selection of photos. Excel spreadsheets were used to compile the extensive experimental data into more comprehensible form.

Aging brought on more and more defects within the worms' neurons. However, even as Day 1 adults, the worms from both strains did show signs of neurodegeneration. At first, very small branches and blebs were witnessed in each strain, and they only appeared in some of the studied specimens. Later, however, greater percentages of worms--eventually 100 percent for the mutant strain--showed decayed characteristics.

The earliest stages of nematode adulthood seemed to lack in branching for the CZ10175 strain of worms, but branching seemed to quickly set in for the TRZ20 worms. Normally, the neurons would appear straight and without deformation. However, the rounded areas known as

blebs along the length of the axons, such as those in **Figure 3, Panel C**, seemed to slowly grow in size as the worms aged. This phenomenon occurred in both strains, though at different rates.

The incidence of blebbing in the worms, as seen in **Figure 3, Panel D**, started to grow as the worms reached "middle age"--about seven to nine days of adulthood.

Imagery

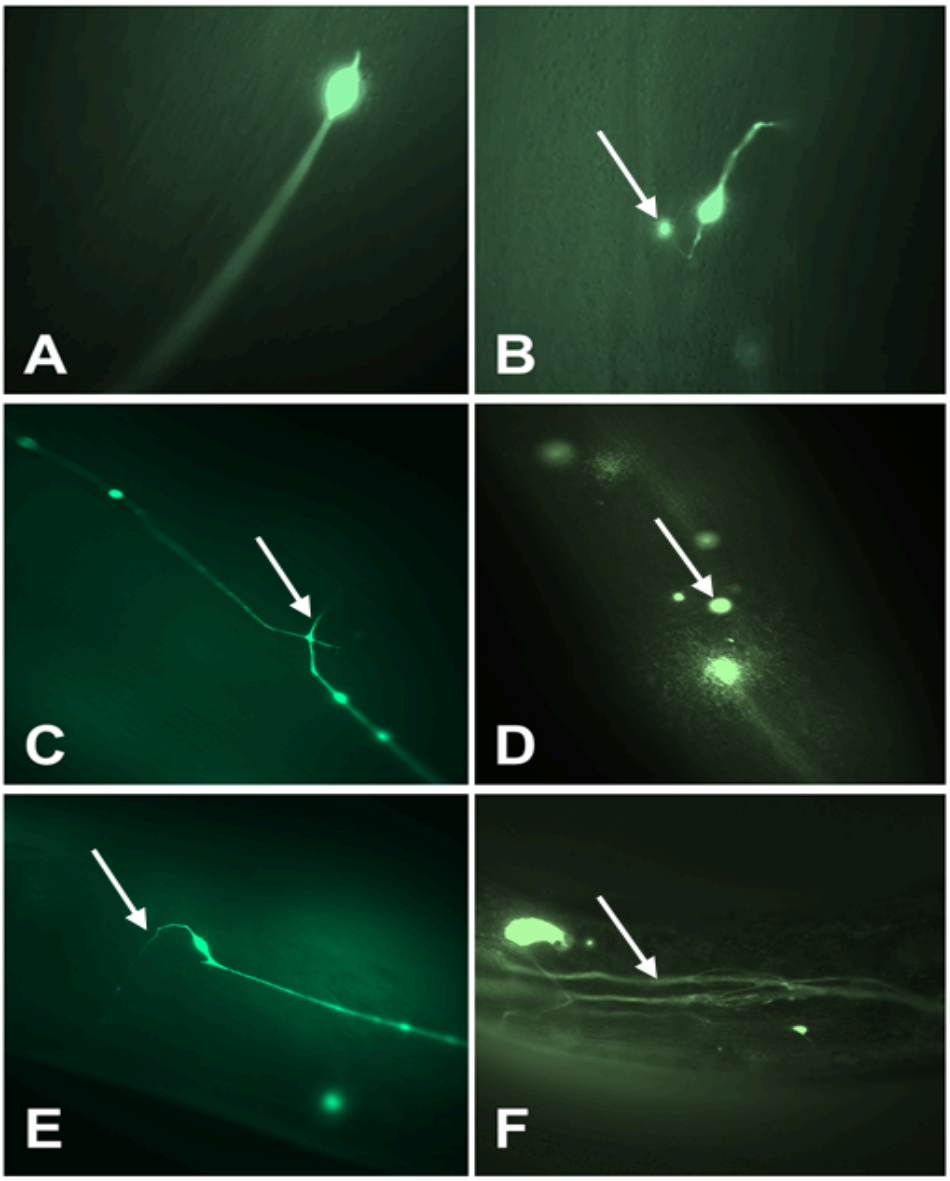

Figure 3. Progression of neurodegeneration in two transgenic strains. Panels **A** through **F** depict the changes witnessed in mechanosensory neurons of the CZ10175 and TRZ20 strains of worms. Gradual increases in neuronal decay can be seen throughout the worms' lifespans. **A**. ALM cell body of a young CZ10175 adult worm. This image depicts a cell body that is considered normal. This is an example of what a neuron looks like before degeneration begins. **B.** CZ10175 adult cell body branch with a bleb. This image shows a cell body found inside a young CZ10175 adult worm. The cell body is seen to have a "tail" with a bleb coming from it. This is an example of early neurodegeneration in a worm. **C.** Blebbing and branching of the PLM neuron. A young adult TRZ20 worm is depicted. Once decay set in, the large nodes and tails coming off the axon began to appear. **D**. Blebbing of the PVM neuron in a Day 7 CZ10175 adult. The largest green mass is the PVM cell body. Working anteriorly from it, several large blebs can be seen. **E**. Branching of the ALM neuron in a Day 13 TRZ20 adult. The ALM cell body depicted has a significant branch extending posteriorly. There is also a minor branch extending off of the anterior end of the ALM body. **F**. Extreme branching of the AVM neuron in a CZ10175 adult. In this image of a Day 18 wild-type adult, the neurodegeneration is extensive. The AVM cell body shows abnormal hypertrophy, and the axon has branched several times.

In the wild-type strain, prevalence of blebbing and branching rose in a similar fashion. However, early on, branching seemed to be the more prevalent defect in the mutant worms, as seen in **Figure 3, Panel C**.

The most severe neuronal decay is represented in **Figure 3, Panel F**. The extent of neurodegeneration undoubtedly caused significant damage and posed a major loss of sensation for the specimen. While disruptions of this extreme were not seen in very many worms, the

handful that were witnessed usually happened later in the lifespan of the nematode for both the CZ10175 and TRZ20 strains.

Graphical Depictions

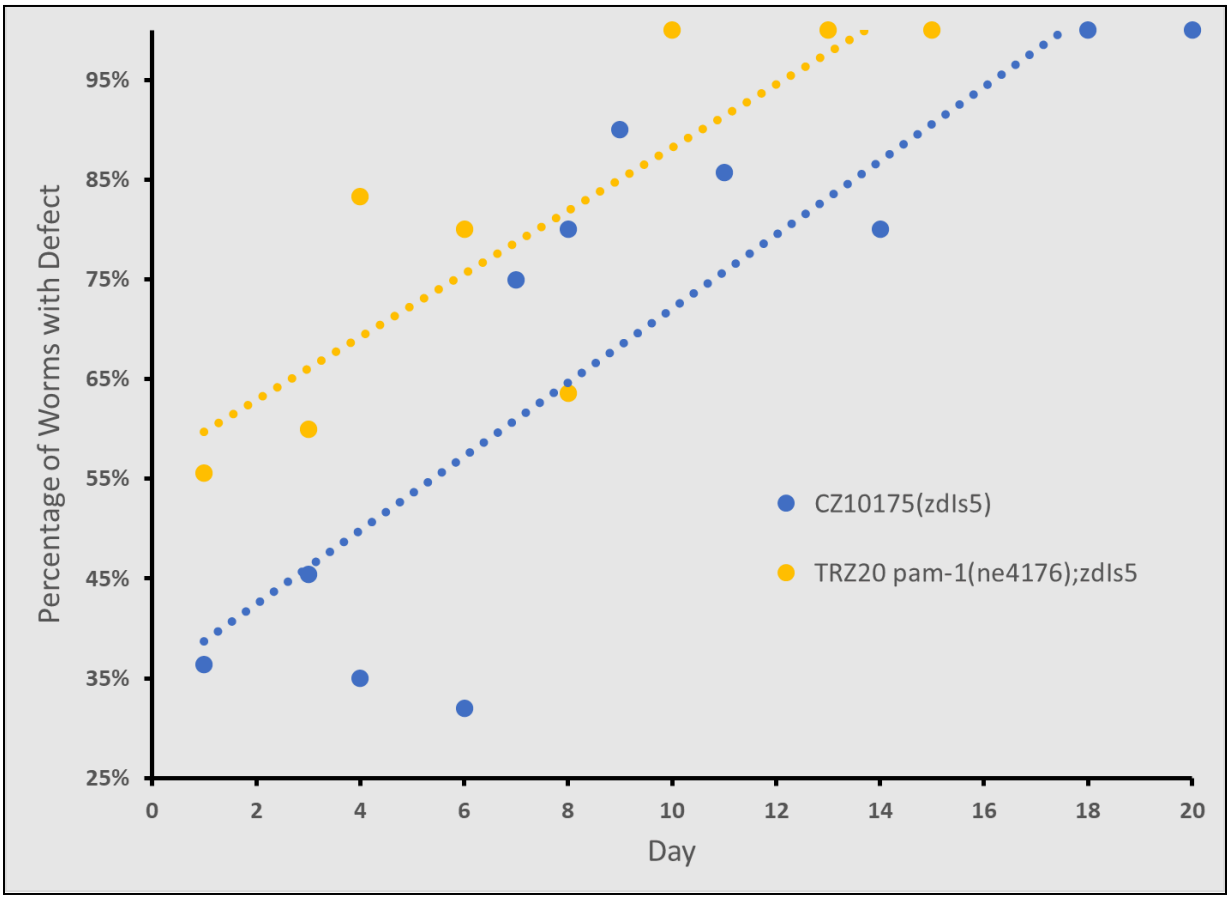

Figure 4. Percentage of worms with any form of neurodegeneration by day. The graph depicts the percentage of worms studied that showed any sign of neuronal defects, including branching and blebbing. After running a regression analysis, CZ10175 and TRZ20 data were found to have a R^2=0.717 and R^2=0.690, respectively.

After running an ANOVA test, the worm-with-any-defects data was not determined to be statistically significant (p=0.320). The following graphs will break down the neuronal defects into two classes, blebbing and branching.

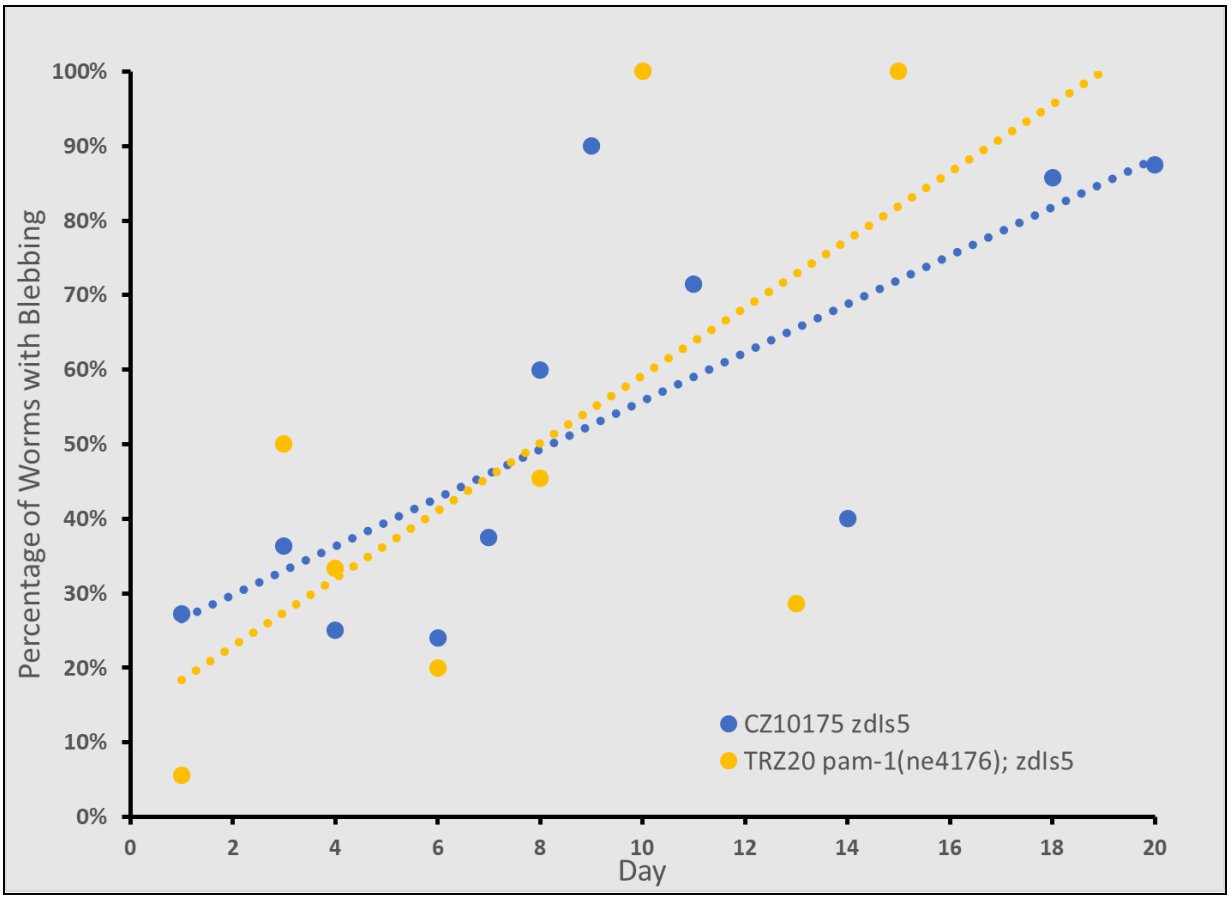

Figure 5. Percentage of worms with axonal or cell body blebbing by day. Typical blebs varied in size, but were primarily found along the lengths of both anterior and posterior mechanosensory axons. After running a regression analysis, CZ10175 and TRZ20 data were found to have a R^2=0.559 and R^2=0.408, respectively.

Utilizing the data collected, an ANOVA test was run on the blebbing data for both strains. Based on this analysis, the blebbing data between the two strains was found to be statistically insignificant (p=0.711). This aligns with the very similar trends seen in **Figure 5**, despite that the TRZ20 worms did peak in degeneration first.

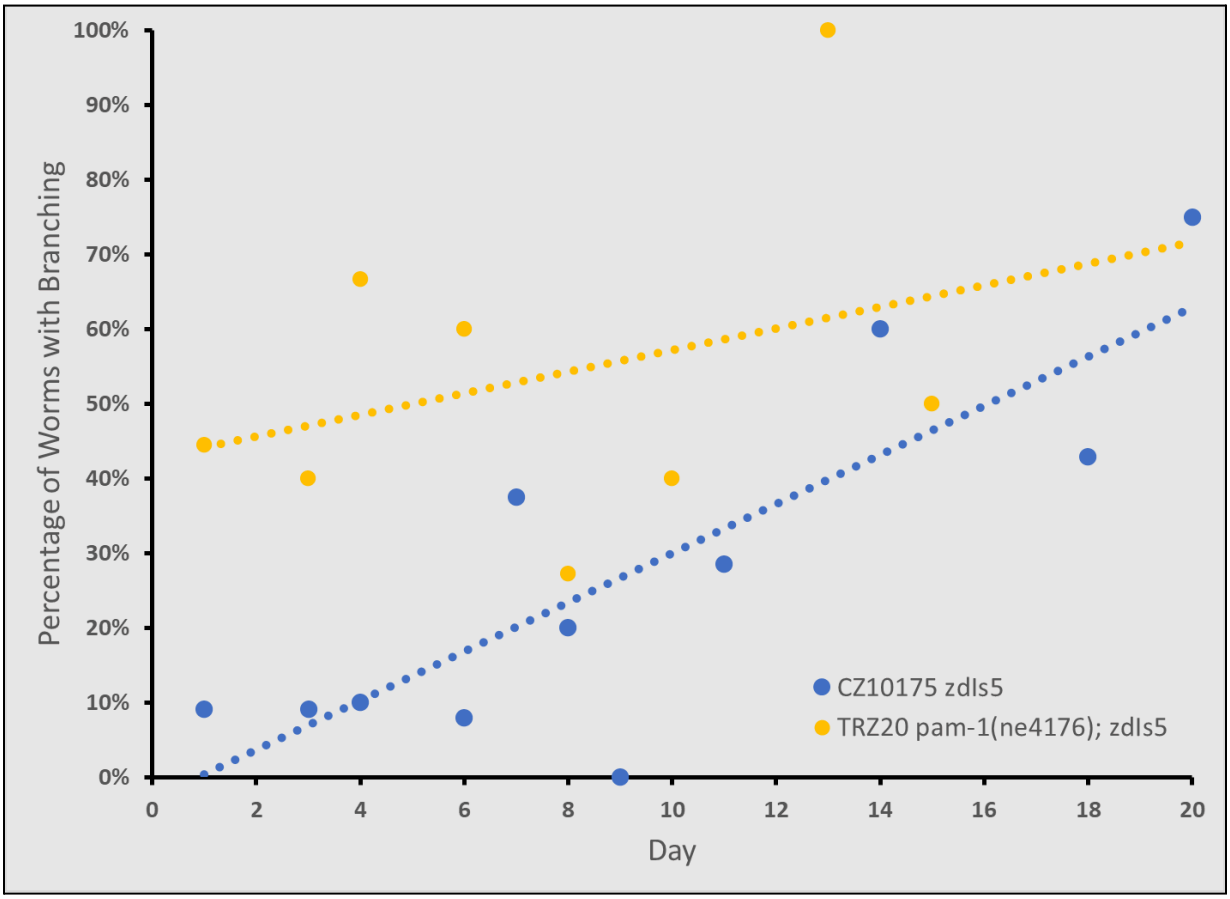

Figure 6. Percentage of worms with axonal or cell body branching by day. Each point of data represents the selected worms for that day. The average number of worms selected for a given day was 11, with a range from 4 to 25. Branching of any kind found on the worms' neurons was considered significant in both strains. After running a regression analysis, CZ10175 and TRZ20 data were found to have a R^2=0.688 and R^2=0.101, respectively.

Using an ANOVA test, the data found on branching in the CZ10175 and TRZ20 worms was analyzed. The results were found to be statistically significant ($p=0.0275$). The F-value was also above the F-crit value.

Discussion

Analysis of data

C. elegans is an immensely useful organism for understanding complexities of neurobiology. Its many features--small, easy to culture, transparent, well-assayed, etc.--make it a classic model organism. Research has been ongoing with this organism for well over a century, and new discoveries are still being made.

This study examines the incidence of neurodegeneration in *C. elegans* and the role of the conserved aminopeptidase PAM-1 in prevention of that neurodegeneration. The imagery and graphs illustrate how the type and severity of defects can differ throughout the worms' lifespans. Initially, very low percentages (an average of 20 percent) of the CZ10175 worms were found to have neuronal decay. Of those that did, typical defects were like those seen in **Figure 3, Panel B**--noticeable, but not shocking. It might be of benefit to study the worms even in their larval stages. This could help answer the question, "When is the very first sign of decay present in a population of studied worms?". The case could be that a very low base level of degeneration is found at almost any age. A single hermaphrodite *C. elegans* can produce around 300 eggs, so there may be imperfections in some of those from the beginning. When considering the defects seen in the TRZ20 worms, they look quite the same in physical appearance. If not for

meticulously keeping track of the worms being studied, as well as understanding some phenotypic differences between the wild-type and mutant worms, one would not perceive a difference when looking solely at the worms' neurons. This is not a surprise considering the invariant cell developments. However, a difference in the amount of degeneration and when it seems to affect a large majority of the population has surely been detected.

Typically, cell bodies will develop large branches and axons will incur some blebbing over time. The TRZ20 worms show similar degradation to the CZ10175 strain, but the mutant specimens seemed to develop their degradation sooner in life than in the wild-type strain, especially as seen in **Figure 4** and **Figure 6**. It was striking to see that TRZ20 worms averaged a 45 percent rate of neuronal branching as Day 1 adults, while CZ10175 worms were found to have an average of less than 10 percent (**Figure 6)** branching at the same time point. **Figure 6** does show that branching in wild-type worms eventually nears the levels found in the mutants, but not until much later in the lifespan. This data was statistically significant ($p=0.0275$). **Figure 5** details a slightly different story. The strains' trend lines representative of neuronal blebbing are quite similar, and the data is nearly indistinguishable. According to the ANOVA, the blebbing results data was insignificant ($p=0.711$). As in the other graphs, the TRZ20 samples did reach 100 percent incidence prior to those of the CZ10175 strain, yet the blebbing results on the whole are inconclusive.

A beneficial overview of the results is found in **Figure 4.** There, any neuronal defects that were witnessed were included in the data set. Like in **Figure 6**, there is a notable difference between the strains when looking at the percentage of worms showing early degeneration. In **Figure 4**, Day 1 mutant adults have neuronal decay rates mirroring those of Day 7 wild-type adults. By Day 10, all TRZ20 worms that were studied showed a neuronal defect of some form,

while it took the CZ10175 worms another eight days of their adult life to reach the same prevalence of neurodegeneration. When considering the results in this manner, a difference between the strains' neurodegeneration is apparent. The trend indicates that biological changes were occurring much sooner and in greater numbers inside the worms with a genetic mutation to the *pam-1* gene. Nonetheless, the only type of defect for which the data is statistically significant is the branching ($p=0.0275$). This might reveal an even deeper characteristic of the PAM-1 aminopeptidase's ability to prevent some forms of neurodegeneration. Alternatively, there may not be enough data points yet with concern to bleb-type neurodegeneration. If more data was collected in the future, the data for other forms of neurodegenerative effects might also become significant statistically. The question is, "Are the differences that are seen in the neurodegeneration between the strains caused directly by the genetic mutation?". The graphs' trend lines clearly show similar slopes, meaning that the increase of neuronal decay is occurring similarly between strains. Yet, higher levels of mutated worms are found with signs of decay much earlier in life (at least for neuronal branching).

Potential for future study

To further elucidate the patterns being observed, it would be helpful to perform lifespan experiments. Anecdotal evidence does suggest that the gene mutation of *pam-1* shortens the *C. elegans*' lifespan, but finding some data concerning this would be necessary. Most *C. elegans* will live up to three weeks, but most of the TRZ20 specimens only lived about two weeks. If lifespan is repeatedly abbreviated in the mutant worms, a couple of possible explanations for this exist. One is that the unusually-rampant onset of neurodegeneration could become lethal early.

The second is the general aging process is sped up on the whole by the *pam-1* mutation, and neurodegeneration just happens to appear sooner as a result (a case of correlation instead of causation). A related path of study that could be used to further investigate this question would be performing functional assays of the worms. By comparing movements and sensory operations of each strain, the physiological implications of the mutation on the organismal level could be better understood.

More data at each step of the worms' lives in general could help clarify the results that have been found thus far. Nonetheless, the lengthy nature of synchronizing populations and screening many worms throughout their adult lives has proven to take a considerable amount of time. For each day that worms were studied, a sample of approximately 11 worms was used. This varied depending on the amount of worms that remained each day. For instance, early on in the data collection for the CZ10175 strain, as many as 25 worms were studied for a given day. On the other end of this, as few as four worms were studied at the end of the lifespan of the TRZ20 strain. This was simply due to the fact that only four mutant worms of the synchronized population were still alive. This made it more difficult to collect as much data on the mutated strain, and this could have clouded some important trends.

One other avenue that could be explored to better understand the accelerated trend of neuronal decay in the TRZ20 worms is assessing certain protein concentrations in the worms. As seen in the experiments with dPSA of the fruit fly, loss of PSA function is conducive to tau protein accumulation. Furthermore, it is known from prior research that tau protein aggregates are well-correlated with neuronal defects (Karsten et al.). It has been seen in multiple models, including fruit flies and transgenic mice, that aggregation of tau leads to tangles of neuronal fibers (Kudo et al.). These are said to be a root cause of some neurological disorders, such as

Alzheimer's disease. PSAs are additionally known to degrade polyglutamine residues (Madabushi et al.) Thus, if antibodies for either tau protein or polyglutamine residue proteins were acquired, these proteins could be bound. By lysing worms of the CZ10175 and TRZ20 strains, and then assaying their proteins with the necessary antibodies and a Western Blot Analysis, concentrations of toxic protein aggregates could be determined. This would give further evidence about the role that PAM-1 plays in neuronal health and toxic protein clearance.

Importance of the *C. elegans* for human applications

The study done here has shown that PAM-1, the PSA homolog of *C. elegans*, may also play a role in destruction of harmful protein aggregates. Mutating the gene definitely impacts the worms, and it appears that this may stem from accelerated neuronal degeneration. It is interesting to note that the other mutated PAM-1 strain, *pam-1(or282)*, is a deletion mutation within the aminopeptidase domain. While this is a much more extensive mutation than the missense mutation used here, many of the detrimental, phenotypic effects of the *or282* strain are mirrored in the *pam-1(ne4175);zdIs5* mutated strain of worms. Thus, the missense mutation must critically destabilize the aminopeptidase domain. Based on this, it would be enlightening to study how the altered PAM-1 interacts with problematic protein aggregates. Studies done by Kudo et al. have shown *in vitro* that a human PSA (hPSA) was able to degrade tau proteins. Considering that the PAM-1 genetic code is 37 percent identical to the genetic code for hPSA, a lot of crossovers could be made (Brooks et al.). By applying the knowledge gained from our study of *pam-1* mutants, and learning if PAM-1 can degrade tau *in vivo*, we would be a step closer to understanding the abilities of PSAs to effectively remove toxic aggregates within organisms. By

gaining this knowledge, therapeutic methodologies could be developed, and, in time, debilitating illnesses like Alzheimer’s disease and Huntington's disease could be slowed or reversed.

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