THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

THE ANALYSIS OF PHENOTYPIC RESPONSES DUE TO POINT MUTATIONS ON SECRETED AND NON-SECRETED SERPINS IN CAENORHABDITIS ELEGANS

PHILIP JAY ROSENSTOCK

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Reviewed and approved* by the following:

Dr. Wendy Hanna-Rose
Associate Professor of Biochemistry and Molecular Biology
Associate Department Head for Undergraduate Studies
Thesis Supervisor

Dr. C.P. David Tu
Professor of Biochemistry and Molecular Biology
Honors Advisor

* Signatures are on file in the Schreyer Honors College.

Abstract

Mutations in the human extracellular serpin, alpha-1 antitrypsin (A1AT) can lead to human disease phenotypes such as liver cirrhosis and emphysema. The most common "Z" variant possesses a mutation that causes accumulation in the endoplasmic reticulum (ER) of liver cells and as a result, a decline of normal plasma levels. Using a *C. elegans* model system, the expression of the Z variant of A1AT (ATZ) mimics the human disease through accumulation in the ER, lack of secretion, low number of progeny, slow development, and shortened lifespan. However, it has not been determined whether these phenotypes are strictly caused by protein misfolding in the ER or whether the expression of ATZ in the cytoplasm can yield similar phenotypes. Therefore, we have chosen to analyze the phenotypic responses caused by forcing wild-type A1AT (ATM) and ATZ into the cytoplasm of the *C. elegans* intestinal cells. We hypothesize that the phenotypes seen in animals expressing ATZ retained in the ER will also be present in transgenic animals expressing the cytoplasmic ATZ. Analyses were based upon the completion of longevity, postembryonic development, and brood size assays. A1AT forced into the cytoplasm was found to produce similar phenotypes to those animals possessing the same extracellular transgene. This finding suggests that regardless of cellular location, the aggregation of mutated A1AT causes these disease phenotypes.

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LIST OF ABBREVIATIONS

A1AT	= Alpha 1-Antitrypsin
ATM	= wild-type extracellular M allele (MR 17.1 integrate line used)
ATZ	= classical extracellular Z mutant with E342K point mutation that forms long-chain-polymer aggregates in the ER (ZR 10.4 integrated line used)
ATM_{CTYO} or ATM Cyto	= wild-type M allele forced into the cytoplasm
ATZ_{CTYO} or ATZ Cyto	= classical Z mutant forced into the cytoplasm to aggregate
P_{nhx-2}	= nxh-2 promoter, which targets expression to intestinal cells
sGFP	= secreted green fluorescent protein
GFP	= non-secreted green fluorescent protein

Introduction

Serine protease inhibitors (Serpins) are a universal family of proteins that express and function in many branches of life, including mammals, plants, viruses and prokaryotes [1]. With more than 1,500 serpin-like genes identified, the serpin superfamily is the largest and most dispersed family of protease inhibitors [2]. In humans, some serpins have been attributed to playing important roles in the control of proteases involved in the inflammatory, complement, coagulation and fibrinolytic pathway [3]. Serpins use a suicide substrate mechanism that irreversibly terminates the enzymatic activity of the protease and thus can perform as a regulator of proteolytic pathways [4]. The suicide substrate mechanism is made possible by the serpin's unique tertiary structure consisting of three β-sheets, eight or nine α-helices, and an exposed reactive center loop (RCL) that presents itself as a pseudosubstrate to the target protease [5, 6]. When a protease interacts with the serpin's RCL, the serpin undergoes an extensive conformational change in order to trap the target protease. Initially, the serpin forms a noncovalent Michaelis-like complex "through residues flanking the scissile bond (P1-P1')" [5]. The active site serine on this scissile bond creates a covalent ester linkage between the protease and the carbonyl of the P1 residue, as well as the cleavage of the peptide bond. At this point, the RCL inserts into the β-sheet of the target protease, distorting its active sight and therefore rendering the protease inactive [5]. Consequently, mutations in the serpin's amino acid sequence in or around the RCL can lead to protein misfolding and dysfunctional molecules with a loss of function [7]. Serpin's possess a region of highly conserved residues that are essential for stability and function know as the shutter region. Mutations in the shutter region can cause the formation of an unstable intermediate that can insert its RCL into the β-sheet of another serpin, creating a

dimer that may lead to the formation of long-chain polymers [8]. Polymers that aggregate within the endoplasmic reticulum (ER) have been found to cause serpin-related diseases associated with either "a clinical phenotype that results from a toxic gain of function due to intracellular protein overload or a disease that results from a loss of function of important protease inhibitors" [8].

Alpha-1 antitrypsin (A1AT) is a human serpin produced in hepatocytes whose main function when circulating throughout the body is to protect the tissues from the enzyme neutrophil elastase, most crucially the tissues of the lungs [9, 10]. A1AT deficiency is a genetic disorder caused by inefficient serpin function that actually results in both the toxic gain of function and loss of function phenotypes. Since first described in 1963 by Carl-Bertil Laurell and Sten Eriksson, there have been over seventy naturally occurring variants of A1AT deficiency identified [8]. One in 2,000 individuals in northern Europe are homozygous for the Z mutation of A1AT deficiency (ATZ), the most sever mutation of A1AT, which results from a Glutamic acid to Lysine substitution at position 342 [8, 11]. ATZ lowers the plasma level of A1AT to a concentration that is 10-15% of an individual who possess the wild-type M allele (ATM) [8]. The Z mutant renders ATZ prone to self-polymerization and aggregation due to its instability of the RCL, which can lead to the toxic accumulation of mutant serpin polymers in the endoplasmic reticulum of liver cells [12, 13]. This buildup of ATZ in hepatocytes has been diagnosed to cause transient juvenile hepatitis in roughly 90% of homozygous individuals with about 2% of these cases leading to liver cirrhosis [14]. Furthermore, the reduction in circulating A1AT through the lungs results in a decline in the amount of inhibitor for neutrophil elastase, identified as the loss

of function phenotype. This consequence of ATZ has been shown to lead to the early-onset of emphysema [15].

There is a clear understanding of the specific disease mutations found in extracellular serpins, which has led to questions regarding the ability of mutated intracellular serpins to aggregate and result in similar disease phenotypes. At this point, there are no known mutations on intracellular serpins that cause serpin related diseases, or serpinopathies. Thus, A1AT variants can be used as a model to investigate possible intracellular serpin-related diseases. By redirecting extracellular ATM and ATZ into the cytoplasm, it can be determine if the ER secretory pathway is required for aggregation of ATZ polymers. If aggregation of ATZ is identified in the cytoplasm of the cell, we can test various phenotypic responses to cytoplasmic aggregation and compare it to aggregation in the ER. Similar phenotypes would allow us to believe that intracellular serpins may also lead to serpinopathies. By using the *C. elegans* as a model system, the mechanisms of cytoplasmic clearance of misfolded serpins can be studied. We can then compare intracellular serpin aggregation to other cytoplasmic protein aggregation disorders such as Huntington's Disease and Parkinson's Disease. Therefore, it is hypothesized that non-secreted ATZ forced into the cytoplasm will result in similar aggregation to secreted ATZ seen in the endoplasmic reticulum.

In previous extracellular serpin experiments, the nhx-2 promoter (P_{nhx-2}) attached to a fluorescent protein has been used to visualize specific proteins of interest in the intestinal cells of live *C. elegans*. Green fluorescent protein (GFP) fusions are a suitable way to visualize protein aggregation while not interfering structurally with the serpins of interest. This intestinal promoter was selected as the primary method of staining the

desired constructs as it is expressed "strongly and uniformly throughout the apical membrane of intestinal cells" in *C. elegans* [16]. The GFP will fluoresce under blue light [17] identifying the location of a protein of interest in the *C. elegans*. To create the desired cytoplasmic alpha 1-antitrypsin, the secretion tag on the protein was mutated. Without the correct secretion tag, A1AT will not be targeted to move through the ER and will be processed in the cytoplasm. Thus, the proteins of interest introduced to the *C. elegans* will remain intracellular.

In order to examine the phenotypic effects of these mutations, three different assays were performed on each line of animals. *C. elegans* serve as a proficient model system for this disease mostly because of their 40% overall gene homology with humans, their swift reproductive cycle, and their short lifespan of approximately twenty-five days [18]. First, longevity experiments were conducted on the *C. elegans* of interest, which include the wild-type *C. elegans* (N2), the secreted and non-secreted GFP controls (sGFP and GFP), and the secreted and non-secreted A1AT transgenic animals (sATM, sATZ, ATM, and ATZ). The longevity assay would help to identify the transgene's affect on the lifespan of the animal. Naturally, longevity is determined by a large number of unknown variables, but accumulated evidence has shown that aging is most affected by a combination of regulatory mechanisms in response to stress, nutrient availability, and environment [19]. Interest in the ability to control aging genetically has increased in the past twenty years due to the discovery that mutation of a single gene can noticeably affect the lifespan of a *C. elegans* [20]. By comparing how long the animal lives to a wild-type animal, one can determine the effects of the mutation on shortening or lengthening its lifespan. We would expect the

animals possessing both sATZ and ATZ to have the shortest lifespan due to the gain of function phenotype caused by the aggregation of long-chain (s)ATZ polymers.

Next, post-embryonic development assays can be executed. These experiments are performed in order to examine the effect of the transgenes on the developmental rate of the animal after hatching from its egg. By doing so, one can determine how quickly each transgenic *C. elegans* will transition through their stages of life from egg to adult. *C. elegans* are an ideal model for this type of study as their life cycle is rapid, normally developing from a fertilized egg through the four larval stages to a mature adult in 3.5 days [21]. We would expect the Z mutation to show significant growth defects characterized by slow development and early larval arrest.

Finally, brood size analyses were performed on each transgenic line, as well as the wild-type animal. The brood size assay allows one to investigate how the number of eggs laid by each worm is altered by the transgenes. By comparing against the wild type (N2) *C. elegans*, we would expect the ATZ transgenics to display the greatest decrease in brood size.

All three of these assays were run simultaneously or separately. However, there were at least three successful trials for each experiment on each line. It is hypothesized that the non-secreted transgenic animals will behave similarly to the secreted transgenic animals through all experiments.

Methods

Design of Constructs

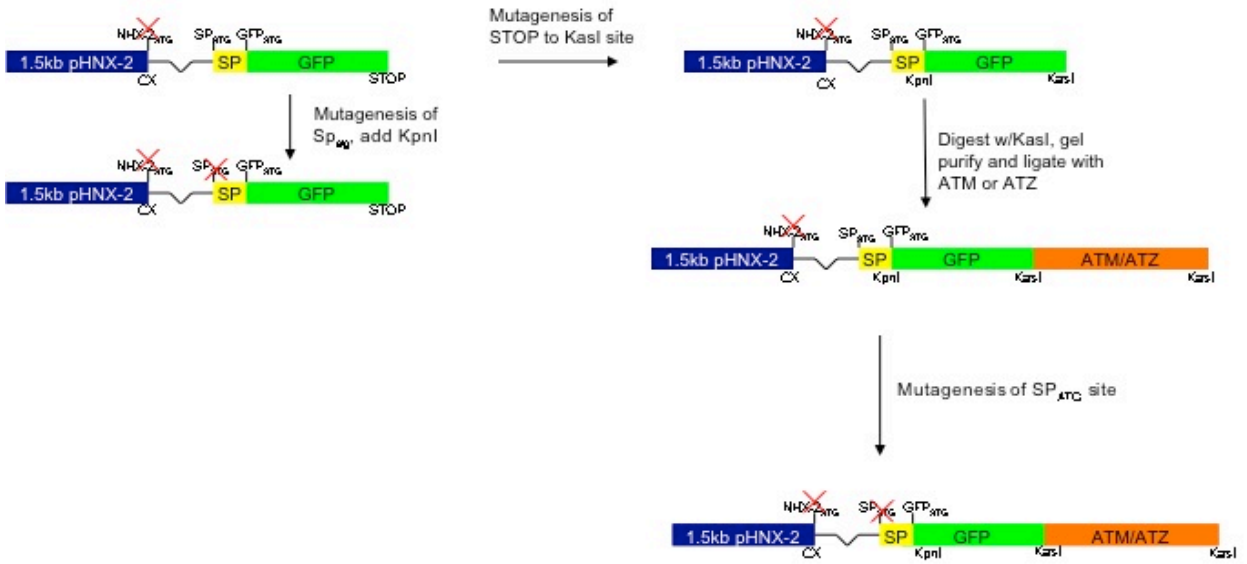


Figure 1: The schematic design used to carry out the creation of the desired constructs.

The design of the constructs was created and performed with the help of the Silverman Lab Group in the Rangos Research Building of the University of Pittsburgh Medical Center's Children's Hospital of Pittsburgh. The positive control, $P_{nhx\text{-}2}$sGFP, was mutated by the enzyme *KpnI* in order to eliminate the secretion tag on the signal peptide fused to GFP. This mutagenisis created the second positive control, $P_{nhx\text{-}2}$GFP, for the non-secreted transgenes.

$P_{nhx\text{-}2}$sGFP also underwent a different mutagensis in order to replace the stop codon of GFP with a *KasI* restriction enzyme site to allow for the insertion of ATM or ATZ. Once ATM or ATZ was ligated to the GFP, the secreted ATM and ATZ constructs were completed. Furthermore, the signal peptide of the ATM and ATZ trangenes was mutated by *KpnI* in order to elminate their secretion tags. This mutagenesis allowed for the construction of the non-secreted ATM and ATZ transgenes.

Longevity Analysis

Longevity assays were performed to analyze the effect of each transgene on a wild-type C. elegans' lifespan. The experiments were performed based on a previously published protocol [22]. However, exactly 25 *C. elegans* at the L4 stage were placed on each 6-cm plate to begin the experiment, instead of 10-15. Furthermore, the addition of FUDR was not utilized to arrest progeny production, as egg laying was not a concern because animals of interest were transferred to new plates every two days. Plates were seeded with 50 µL of OP50 bacteria and worms were grown in a 20°C incubator [23]. Analysis took place daily and the worms were scored as alive, dead, dried, or missing. All dead animals were removed from the plate by a platinum pick. An animal is identified as dead when it has no response to touch by the platinum pick [22]. Dried and missing worms were considered censored animals when scored. At each time point, the number of living animals is measured. Animals were transferred to new plates every 2 days until the cessation of egg laying [23]. The transfer of animals then only took place when food was depleted. Wild type animals have a lifespan of approximately 18 days [22]. At the end of the experiment, data is analyzed using a Kaplan-Meier survival curve.

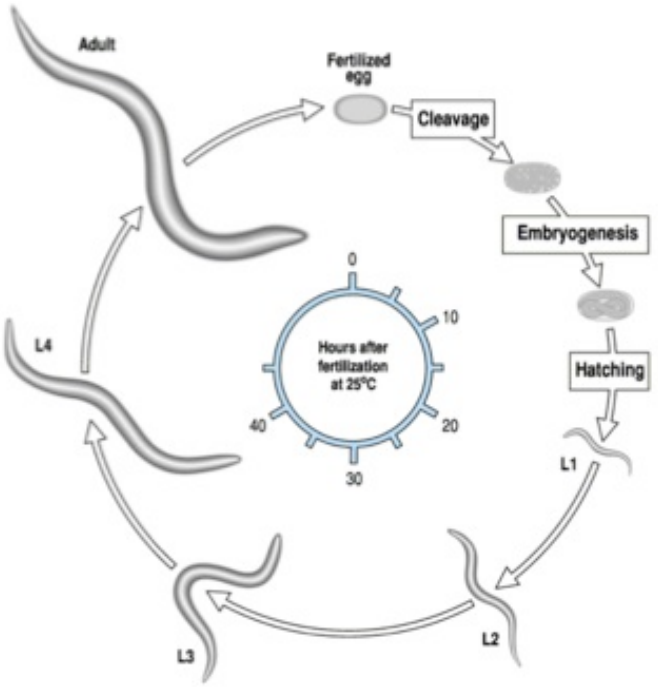


Image 1: The lifecycle of a *C. elegans*

Postembryonic Development Analysis

Postembryonic development assays were carried out to determine any variance in the animal's growth and development due to the introduction of the different transgenes. Protocols were performed similarly to previous post-embryonic development assays [21]. Twenty egg laying adult worms were placed on a 6-cm agar plate seeded with OP50 [21]. These adult hermaphrodites were allowed to lay eggs for 2 hours [24]. All adults were then removed from the plates and eggs were placed at 25°C to develop for 48 hours. Following the time allotted for development, the animals were scored based upon their developmental stages. A percentage was calculated based upon the number of animals at each developmental stage [24].

Brood Size Analysis

In this set of experiments N2 and transgenic animals were grown on 6-cm plates seeded with OP50 bacteria, similar to previous protocols [23]. To begin the experiment, individual animals in the L4 stage were placed on separate plates. Every 24-hours the parental animals were transferred to a new plate until the cessation of egg laying. Each plate was kept at 20°C during the experiment and for 48 hours following the removal of each adult animal. After 48 hours, plates were scored for the number of F1 animals present [23]. For every individual animal, the total number of eggs was calculated and identified as the total brood size.

Results

Transgenic Lines

Trasgenic animals expressing all constructs were generated by injecting 20 ng/µL of the plasmid DNA, along with 20 ng/µL of co-injection marker and 60 ng/µL of pBluescript into the gonads of wild-type (N2) young adult hermaphrodites. In order to increase the concentration of injected DNA and the probability of successful injections, vector DNA in the form of pBluescript, also called carrier DNA, is utilized.

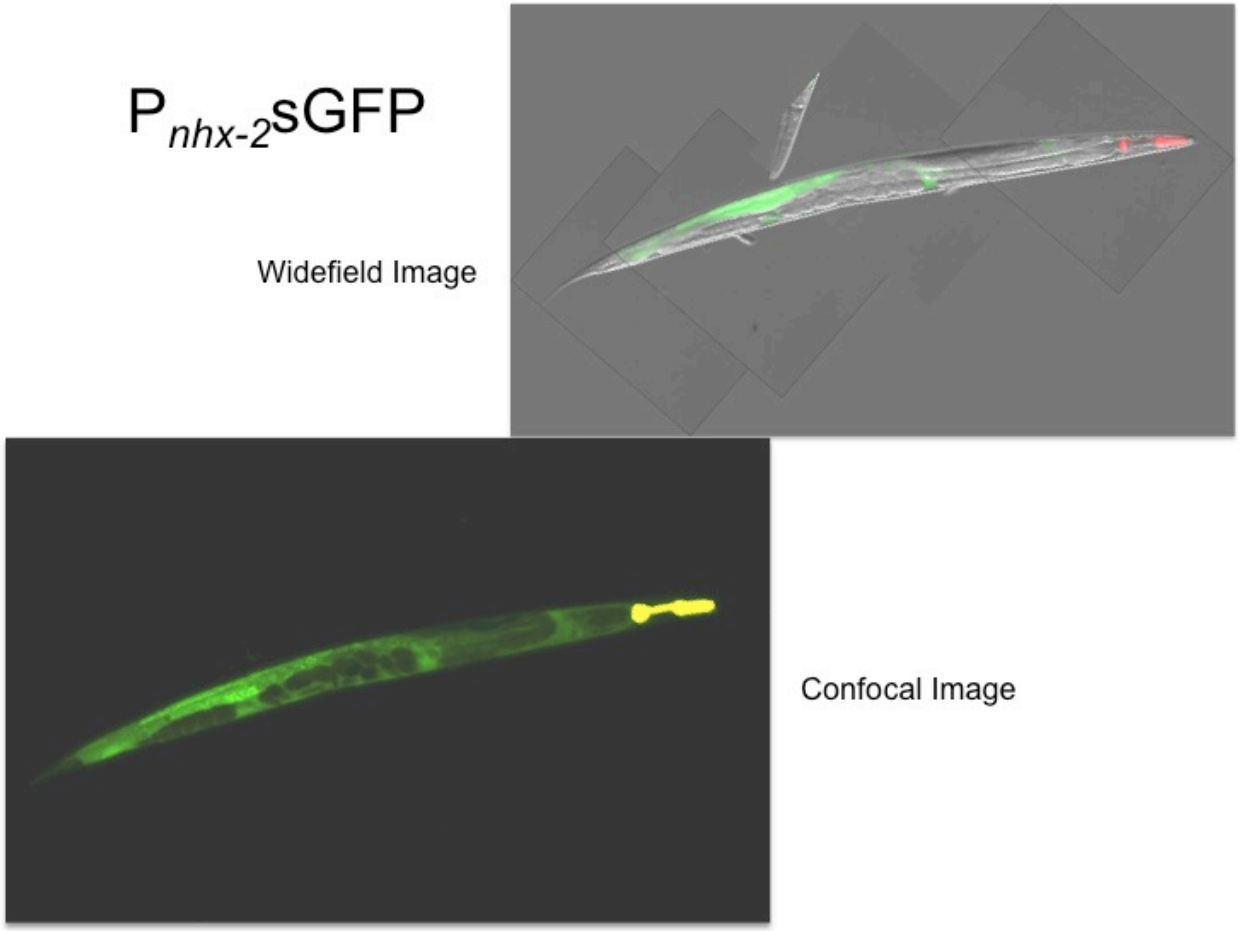


Image 2: A transgenic *C. elegans* possessing secreted GFP targeted for intestinal cells by the nhx-2 promoter. The image shows the secreted green fluorescent protein throughout the pseudocelomic space (along the exterior-most portions of the worm body). The red staining of the pharyngeal muscle in both images (although the confocal image appears yellow) is caused by a myo-2::mCherry co-injection marker.

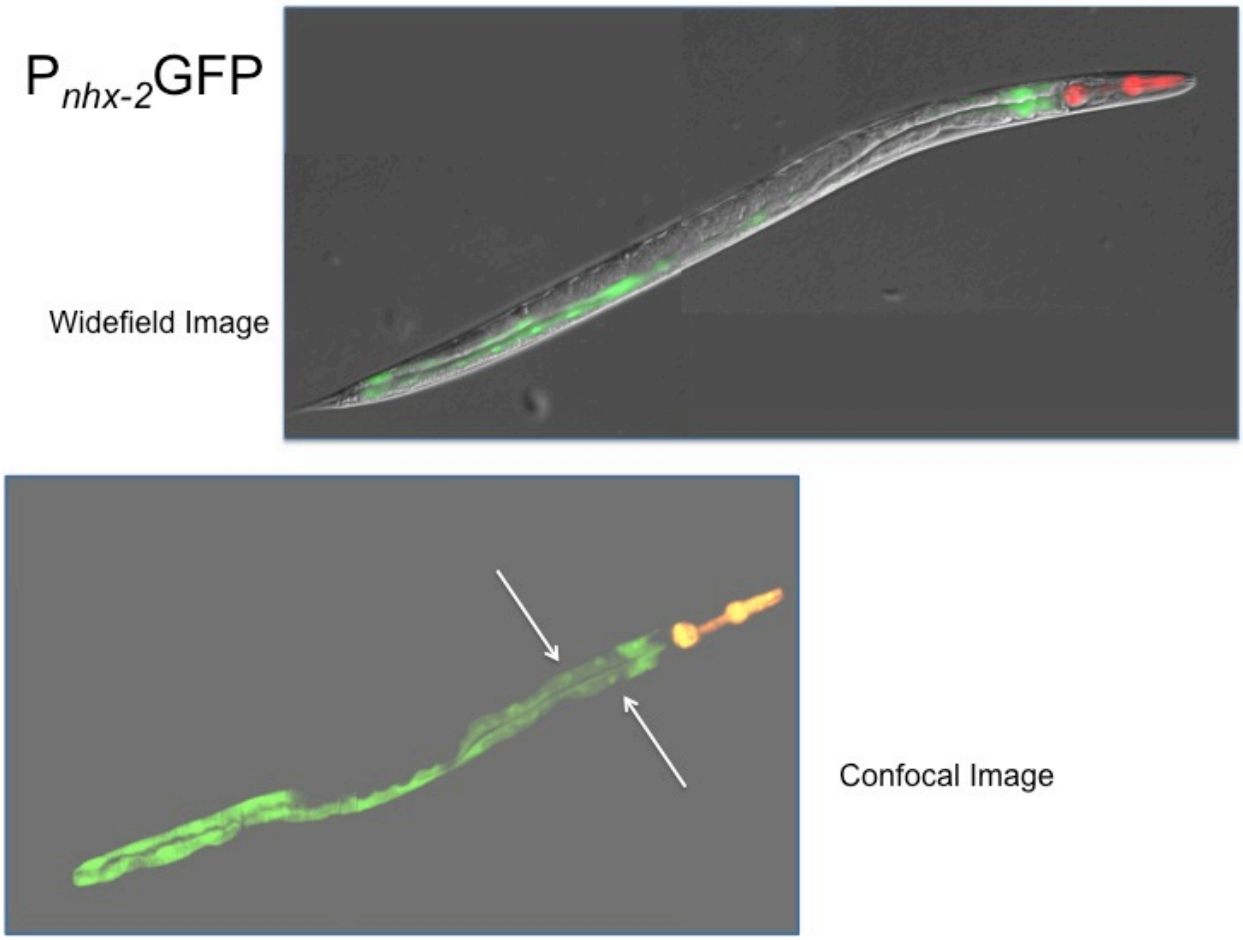


Image 3: A transgenic *C. elegans* possessing non-secreted GFP targeted for intestinal cells by the nhx-2 promoter. Because the protein lacks a secretion tag on the green fluorescent protein, it is retained in the cytoplasm of the intestinal cells and is further identified by some anterior nucleus staining (shown by the arrows).

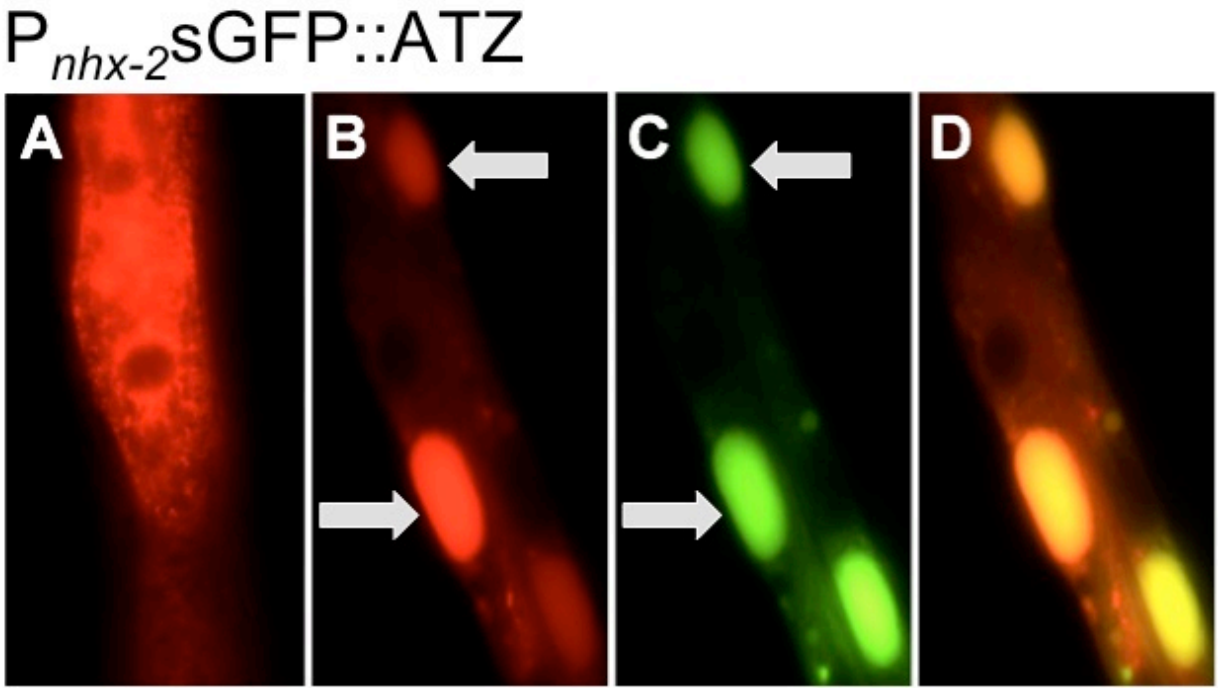


Image 4: A transgenic *C. elegans* with A1AT possessing the "Z" mutation (Glutamic acid to Lysine at position 342) that is fused to secreted GFP targeted for intestinal cells by the nhx-2 promoter (sATZ). Panel A shows a *C. elegans* injected with only P_{nhx-2}KDEL::dsRed marker that targets dsRed to the ER of intestinal cells. Panels B, C, and D show an animal injected with ATZ and co-injected with the P_{nhx-2}KDEL::dsRed marker that possesses the aggregates in the Endoplasmic Reticulum that are produced as a result of the mutation (identified by the arrows). The co-localization seen in image D confirms that the aggregates are found in the endoplasmic reticulum.
Image – (Pak 2011)
* An image of the ATM fused to secreted GFP and co-injected with KDEL-dsRed would show co-localization of green and red staining in the Endoplasmic Reticulum, but possesses no aggregates.

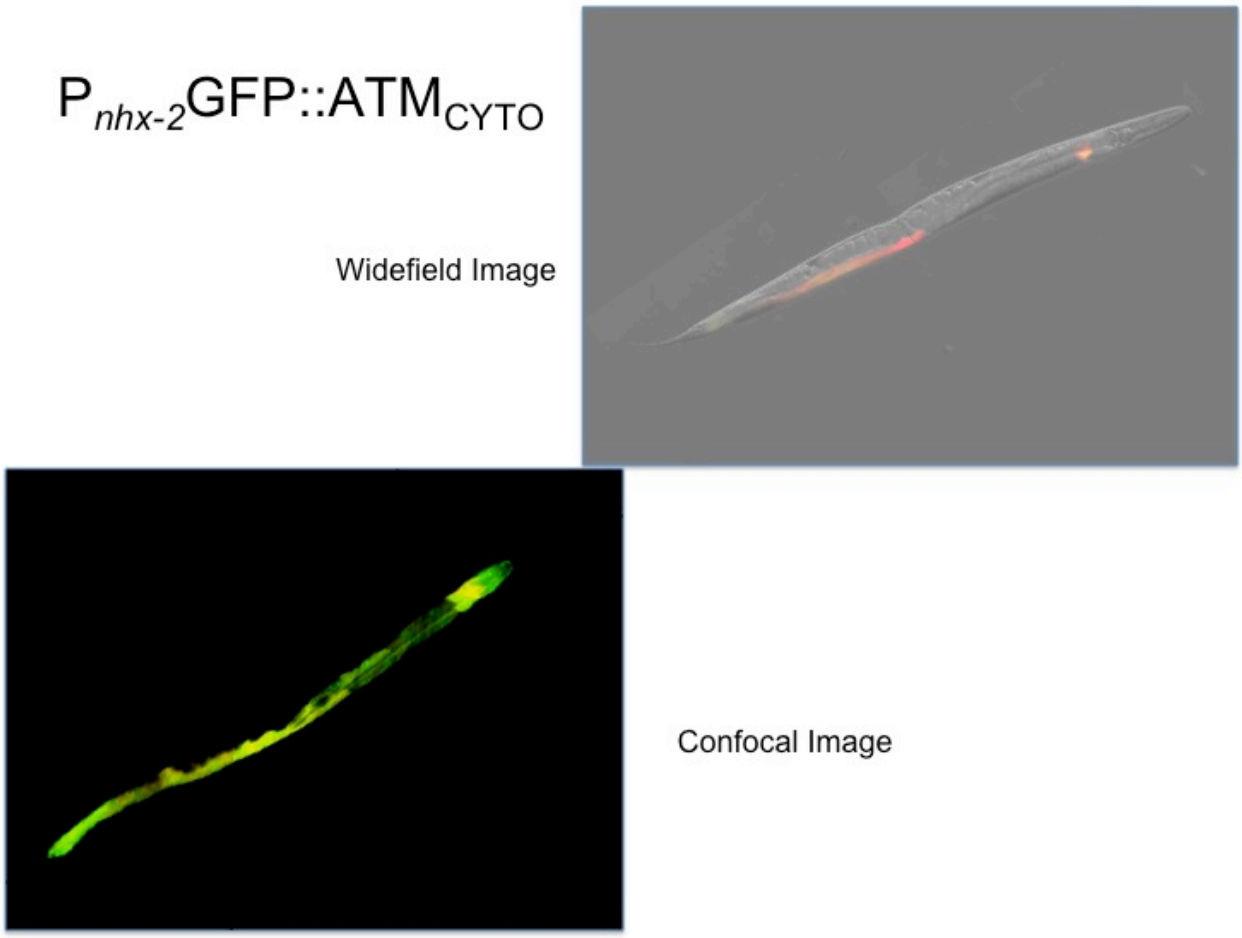


Image 5: A transgenic *C. elegans* possessing A1AT fused to non-secreted GFP targeted for intestinal cells by the nhx-2 promoter (ATM). Because the protein lacks a secretion tag on the green fluorescent protein, it is retained in the cytoplasm of the intestinal cells. The confocal image shows a maximum intensity projection of the confocal Z slices. The transgenic *C. elegans* was also co-injected with the P_{nhx-2}KDEL::dsRed marker that targets dsRed to the ER of intestinal cells.

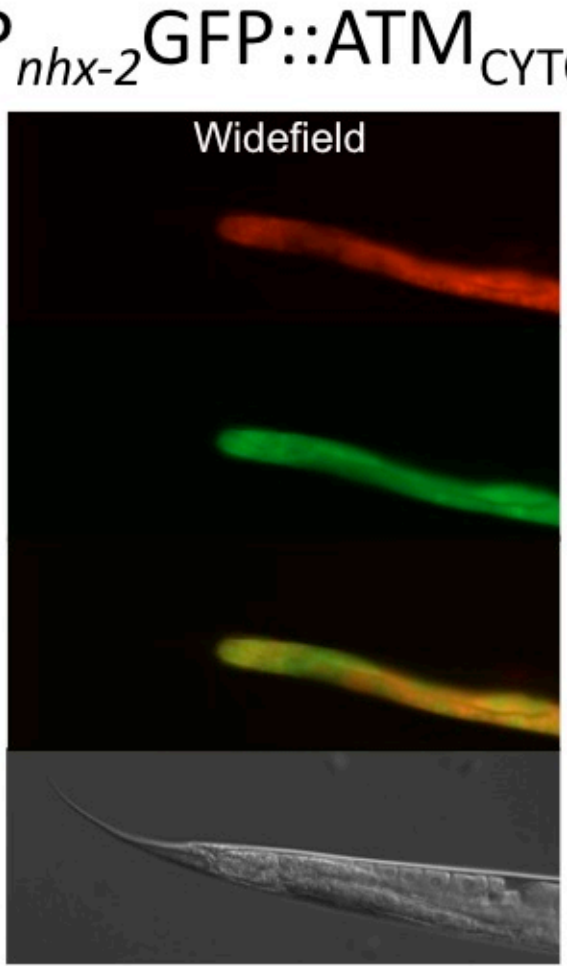


Image 6: A higher magnification of the transgenic *C. elegans* injected with ATM. The transgenic *C. elegans* was also co-injected with the P_{nhx-2}KDEL::dsRed marker that targets dsRed to the ER of intestinal cells. The above images separate the expression of the GFP and KDEL-dsRed, as well as provide picture of the combined expression. The widefield image shows that the GFP does not co-localize with the KDEL-dsRed, proving that the GFP is localized in the cytoplasm and the KDEL-dsRed in the ER.

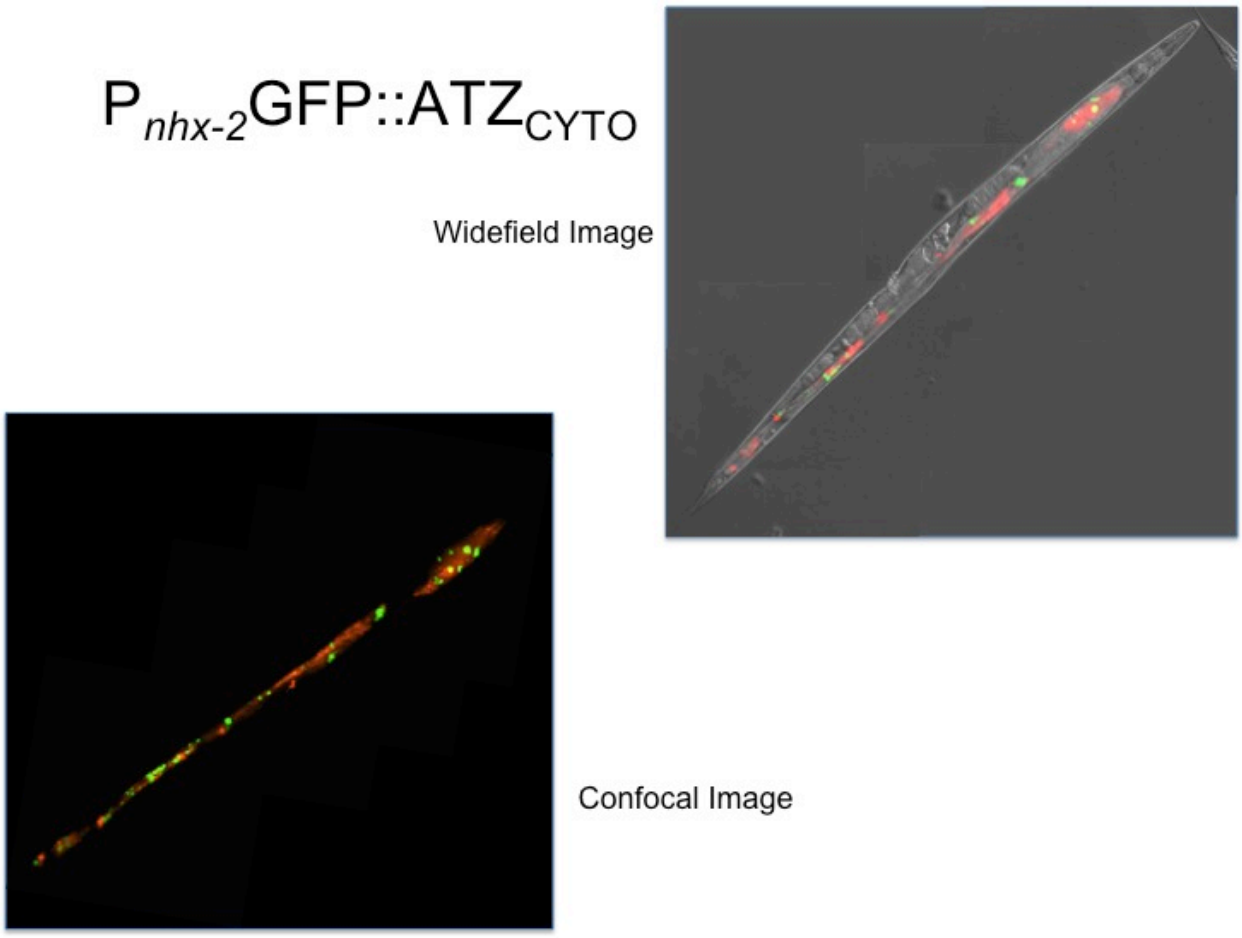


Image 7: A transgenic *C. elegans* injected with A1AT possessing the "Z" mutation (Glutamic acid to Lysine at position 342) that is fused to non-secreted GFP targeted for intestinal cells by the nhx-2 promoter (ATZ). Because the protein lacks a secretion tag on the green fluorescent protein, it is retained in the cytoplasm of the intestinal cells. The transgenic *C. elegans* was also co-injected with the P_{nhx-2}KDEL::dsRed marker that targets dsRed to the ER of intestinal cells. Notice the aggregates produced as a result of the mutation that are stained by GFP throughout the intestine of the animal and do not co-localize with the KDEL-dsRed staining.

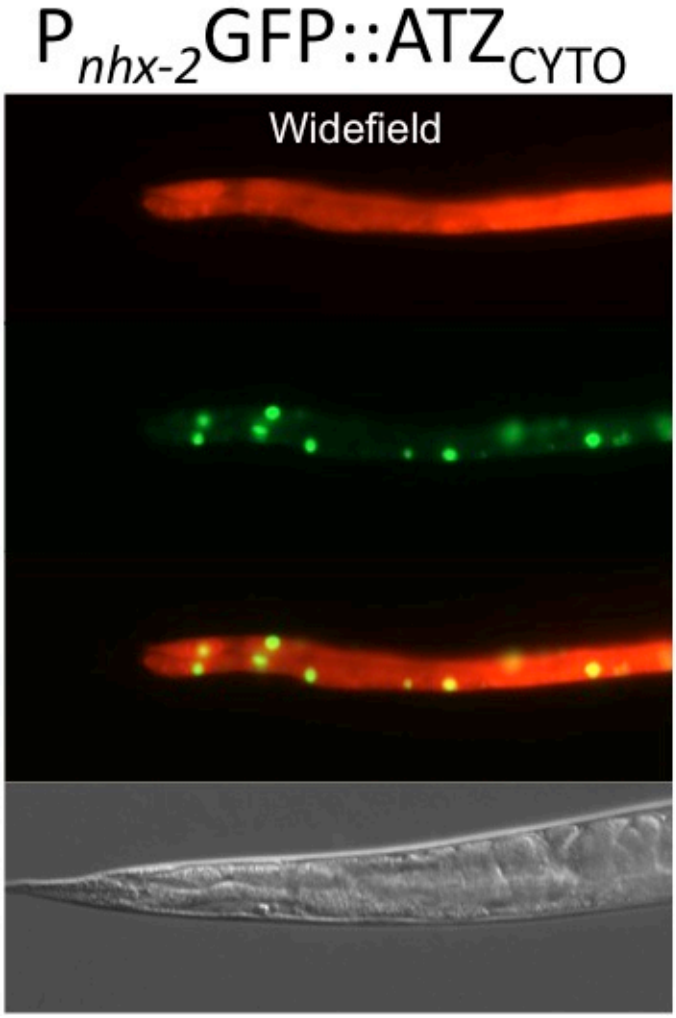
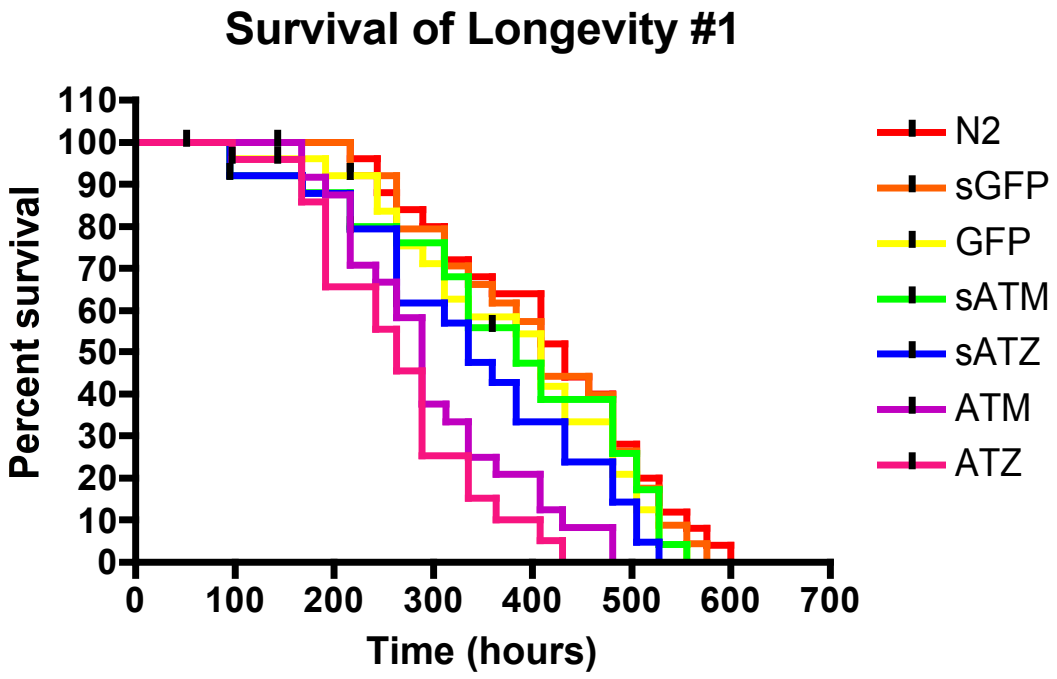
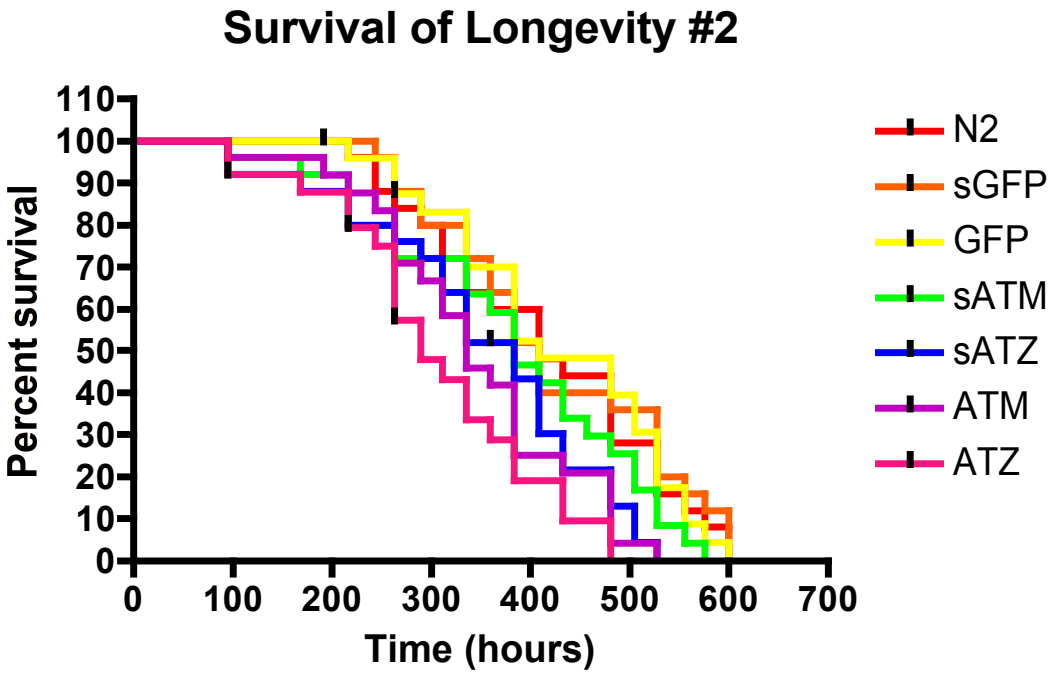


Image 8: A higher magnification of the transgenic *C. elegans* injected with ATZ. The transgenic *C. elegans* was also co-injected with the P_{nhx-2}KDEL::dsRed marker that targets dsRed to the ER of intestinal cells. The above images separate the expression of the GFP and KDEL-dsRed, as well as provide picture of the combined expression. The widefield image shows that the GFP stained aggregates do not co-localize with the KDEL-dsRed, proving that the mutated A1AT aggregates in the cytoplasm. These are very substantial images because they show that the serpin does not need to pass through the ER to aggregate, as previously believed.

Experimental Assays

Longevity, postembryonic development, and brood size analyses were performed in order to test the animals' response to the introduction of secreted and non-secreted A1AT variants. Three longevity assays were successfully completed and their data was then combined into a "total longevity" graph that combined all curves. Experimental analysis was performed on the curve of combined data. The averages of three postembryonic development assays and six brood size analyses are presented in Figures 3 and 4, respectively. Statistical analysis of the postembryonic development is presented in a table below its corresponding figure.




Survival of Longevity #2
Percent survival
110
100
90
80
70
60
50
40
30
20
10
0
0
100
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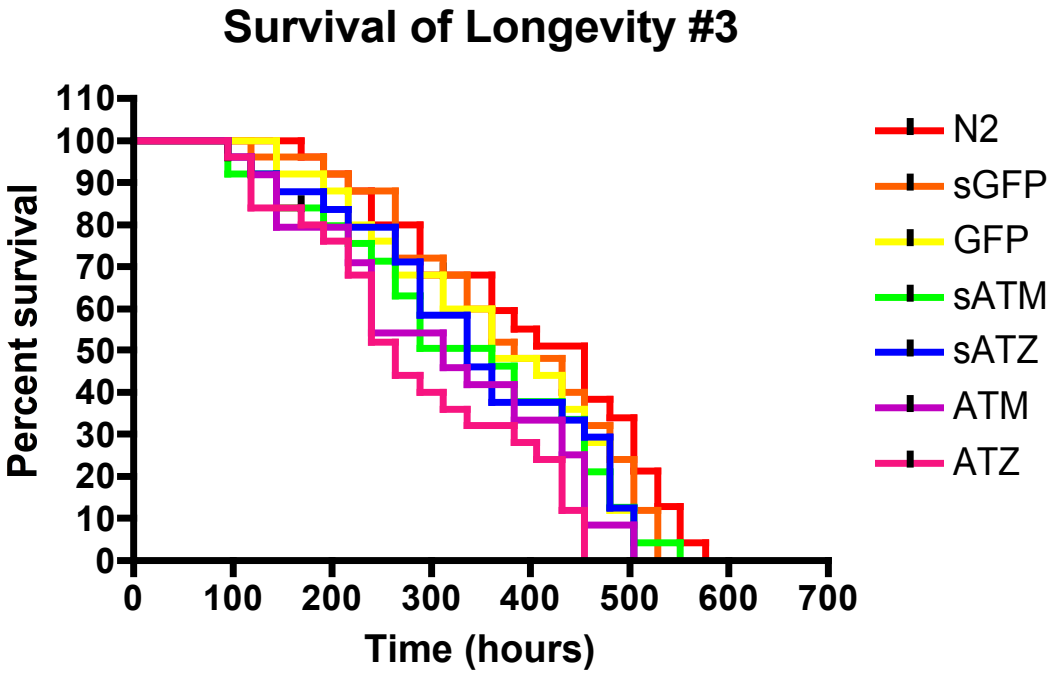
500
600
700
Time (hours)
N2
sGFP
GFP
sATM
sATZ
ATM
ATZ


Survival of Longevity #3
Percent survival
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100
90
80
70
60
50
40
30
20
10
0
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100
200
300
400
500
600
700
Time (hours)
N2
sGFP
GFP
sATM
sATZ
ATM
ATZ

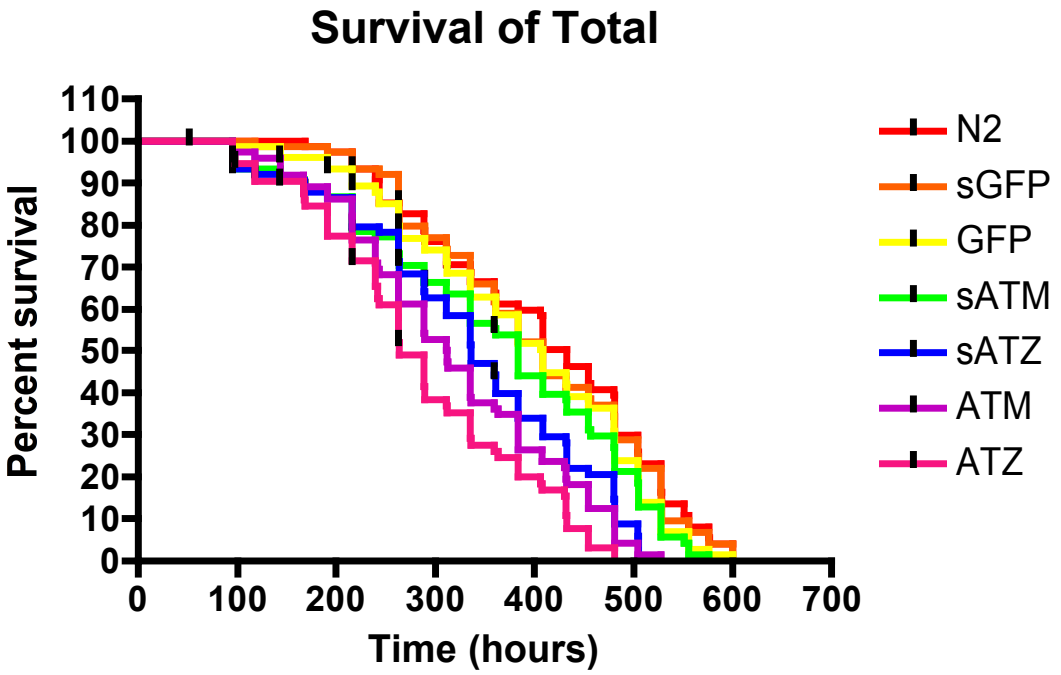

Figure 2: The above figure displays the percentage of the starting population surviving over the duration of time in hours. The assay was performed on twenty-five L4 animals placed on a 6-cm plate seeded with OP50 and stored at 20°C.

Table 1: Analysis of Combined Longevity Assays

Construct	Median Survival	P-value vs N2[a]	Significance[b]
N2	432.5		
sGFP	408.5	0.7242	ns
GFP	408.5	0.1721	ns
sATM	383.5	0.0237	*
sATZ	336.0	0.0003	***
ATM	311.5	<0.0001	***
ATZ	264.0	<0.0001	***

The above table shows the analysis of the combined longevity assay for all transgenic animals versus the N2 animals. Statistical analysis was based upon a Logrank Test for the Kaplan-Meier Survival Curve.

[a] = Logrank analyses to determine p-values were performed by Prism 4 software

[b] * = degree of statistical significance identified by Prism 4. A greater number of asterisks = a larger degree of statistical significance.

Table 2: Comparison of Secreted and Non-Secreted A1AT Longevity Assays

Comparison	P-value[a]	Significance[b]
sATM vs sATZ	0.0219	*
ATM vs ATZ	0.0406	*

The above table displays the Logrank test comparing the combined longevity assay for the secreted and non-secreted variations ATM and ATZ. The p-values are based upon the Logrank for the combined Kaplan-Meier Survival Curve.
[a] = Logrank analyses to determine p-values were performed by Prism 4 software
[b] * = degree of statistical significance identified by Prism 4. A greater number of asterisks = a larger degree of statistical significance.

All three of the longevity assays displayed similar trends in median survival for each line. When each longevity experiment was combined to create a total longevity assay, we found that all A1AT animals were significantly different than the N2 animals. We also observed that both sATZ and ATZ animals showed a decreased lifespan that was significantly different than sATM and sATZ.

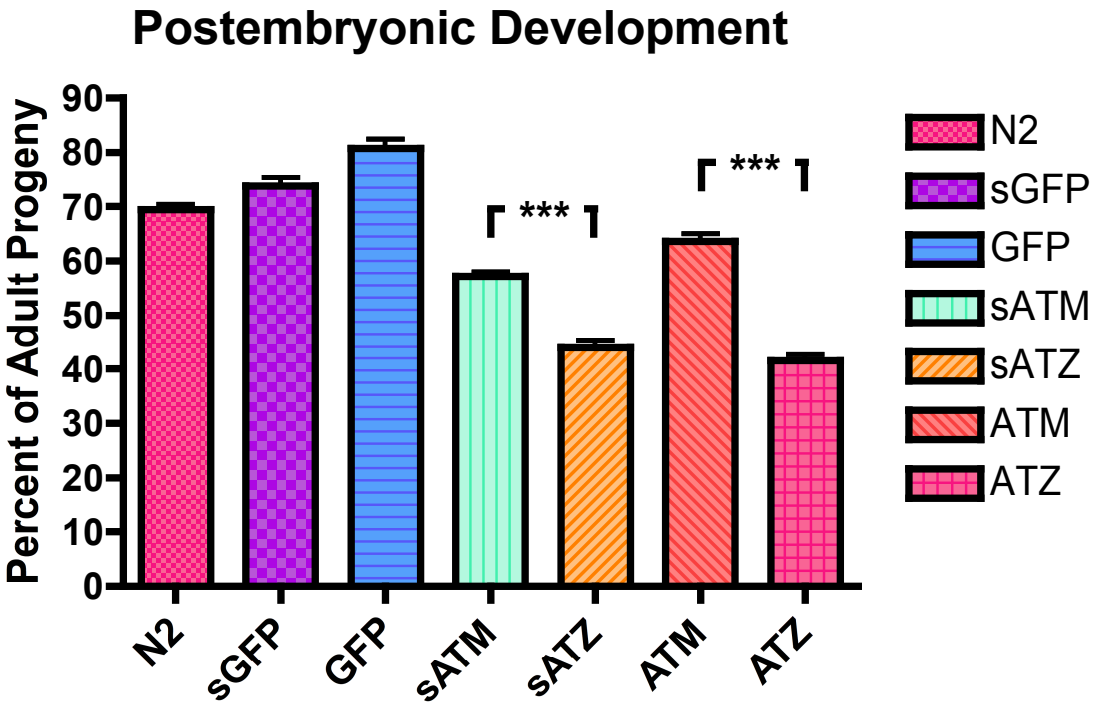

Figure 3: The analysis of postembryonic development in all of the tested transgenic *C. elegans*. The table displays the percent of an animal's progeny at the adult stage after 48 hours of development at 25°C. Each transgenic line contains the average percentages for three animals assayed.

Table 3: Analysis of Combined Post Embryonic Development

Construct	Percent of Adult Progeny	P-value vs N2[a]	Significance[b]
N2	69.45 ± 0.8811		
sGFP	73.83 ± 1.541	0.0689	ns
GFP	80.69 ± 1.710	0.0043	**
sATM	57.21 ± 0.6658	0.0004	***
sATZ	44.05 ± 1.306	<0.0001	***
ATM	63.62 ± 1.389	0.0239	*
ATZ	41.56 ± 1.067	<0.0001	***

The statistical analysis of the postembryonic development assays. Two-tailed student's t-tests were used to determine p-values were performed by Prism 4 software. The p-values were then used to analyze the degree of statistical significance identified by Prism 4. A greater number of asterisks represent a larger degree of statistical significance.

Table 4: Comparison of Secreted and Non-Secreted A1AT Post Embryonic Development

Comparison	P-value[a]	Significance[b]
sATM vs sATZ	0.0009	***
ATM vs ATZ	0.0002	***

The statistical analysis of secreted and non-secreted A1AT animals through data collected during the postembryonic development assays. Two-tailed student's t-tests were used to determine p-values were performed by Prism 4 software. The p-values were then used to analyze the degree of statistical significance identified by Prism 4. A greater number of asterisks represent a larger degree of statistical significance.

The postembryonic development assays display a delayed progression through developmental stages for both secreted and non-secreted ATM and ATZ animals. Based upon the results, there were a greater percentage of animals in the adult stage for *C. elegans* that did not possess any form of the A1AT DNA. Those animals that did possess some form of A1AT showed an increased percentage in the early developmental stages. After performing a two-tailed student's t-test, there was a significant difference between sATM versus sATZ, as well as ATM versus ATZ.

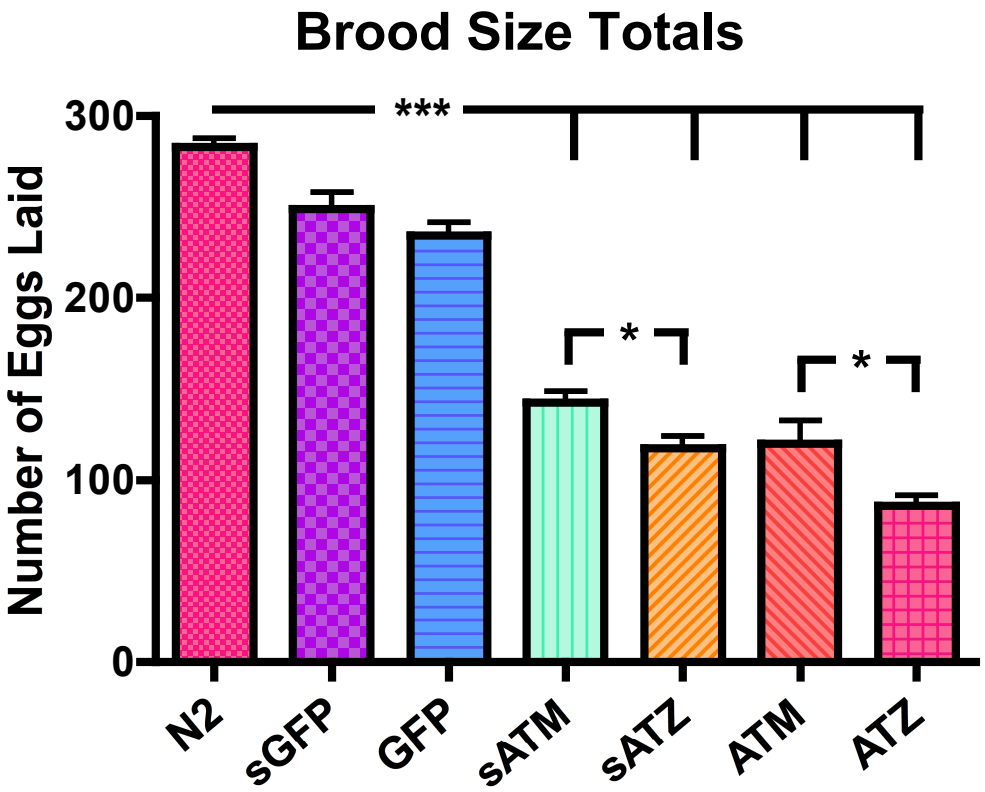

Figure 4: The above graph displays the average brood size for the secreted and non-secreted transgenic animals. Six brood size assays were performed on an individual animal of each transgene at 20°C. Animals were transferred to new plates every 24-hours until all eggs were laid. Two-tailed student's t-tests were utilized to determine p-values using Prism 4 software. The p-values were then used to determine the degree of statistical significance identified by Prism 4. A greater number of asterisks represent a larger degree of statistical significance.

The results of the brood size assays show a clear decrease in egg production for A1AT transgenic animals. Through a two-tailed student's t-test, a strong statistical significance was found when comparing the N2 animals to both secreted and non-secreted A1AT transgenic animals. ATZ and ATM animals did significantly differ from one another, as ATZ possessed a lower brood size. A similar difference was observed when comparing sATZ to sATM, showing that sATZ had a significantly lower brood size as well.

Discussion and Conclusions

In previous experiments, it was identified that secreted A1AT variants (sATM and sATZ) display similar results to those found in these assays. The inclusion of cytoplasmic A1AT transgenic animals (ATM and ATZ) have allowed us to conclude that there is a similar phenotypic response expressed in animals possessing the non-secreted A1AT to their secreted counterpart. In the longevity assays, we found that the ATZ caused a reduced lifespan compared to ATM, similar to the comparison of sATZ versus sATM but ATM and ATZ actually had the shortest lifespan of all animals tested. Through the Logrank test of the Kaplan-Meier Survival Curve for the total longevity data, we found that the survival of both sATZ and ATZ were significantly different than sATM and ATM, respectively. However, we cannot compare sATZ to ATZ and sATM to ATM because the secreted variants are integrated transgenic lines that possess different levels of expression than the non-secreted A1AT transgenic animals. Because intracellular aggregates found in ATZ animals were observed and confirmed to exist in the cytoplasm of the intestinal cells (see images 6 & 7), it is possible that the cytoplasmic protein processing may not function as efficiently as the ER, and thus cytoplasmic aggregation actually causes the animal to become slightly sicker. Still, it is also possible that ATM and ATZ transgenic animals had a much greater level of expression compared to sATM and sATZ, which resulted in the overall shorter lifespans.

The postembryonic development assays also displayed similar results. We observed strong significant difference when comparing sATM to sATZ and ATM to ATZ. All A1AT animals were found to possess a slowed progression through development compared to N2, but that is most likely due to the introduction of foreign DNA to be processed by the animal. As expected, both sATZ and ATZ animals exhibited impeded development when

compared to their ATM counterpart. The aggregation of cytoplasmic ATZ long-chain-polymers clearly inhibits the development of *C. elegans* just as aggregation of polymers in the ER. Thus, as predicted, non-secreted ATZ aggregation displays a similar developmental phenotype to secreted ATZ.

Furthermore, the brood size assays followed a similar trend. All variants of secreted and non-secreted A1AT transgenic animals experienced a statistically significant decline in the number of eggs laid when compared to the N2 *C. elegans*. As expected, the sATZ and ATZ animals produced a smaller brood size than their ATM counterpart, establishing that aggregation in the ER versus aggregation in the cytoplasm creates a similar decrease in the *C. elegans* number of progeny. Moreover, the ATZ animals produced lower numbers of progeny than sATZ. This may be due to an increased level of expression in ATZ, or due to the fact that cytoplasmic aggregation actually inhibits the brood size at a greater extent than aggregation in the ER.

In summary, both sATZ and ATZ show a reduced lifespan, development, and brood size in comparison to sATM and ATM, respectively. It is interesting to note that although the comparison between sATZ and ATZ is not possible, the difference between the two secreted A1AT transgenic animals and the non-secreted A1AT animals possess similar trends. For example, the median survival for sATM was 383.5 hours while sATZ was 336.0 hours. The median survival for ATM was 311.5 hours while ATZ was 264.0 hours. In both cases, the Z variant had a decreased lifespan by about 50 hours. The same types of trends are observed in the postembryonic development and brood size assays. This leads us to believe that not only is cytoplasmic aggregation possible, but it results in truly similar phenotypes to aggregation in the ER. The reduction in lifespan, postembryonic

development, and brood size is caused by aggregation, therefore diminishing the idea that it was ER stress caused by aggregation that yielded the previously examined results.

As previously stated, it is possible that the ATM and ATZ transgenic animals could possess a greater expression than sATM and sATZ, resulting in a greater degree of reduction through all assays. However, it could be due to the fact that cytoplasmic aggregation occurs at a higher degree because its degredation pathway (ex. Autophagy) does not handle to misfolded proteins as well as the ER (Endoplasmic Reticulum Associated protein Degredation). This may in fact lead to discoveries as to how the cell handles cytoplasmic aggregation of proteins in cells other than neurons, which have already been examined.

This experiment has served as a model for intracellular serpin aggregation. The next steps to confirming these observations would be to all for the comparison of sATZ to ATZ and sATM to ATM. This analysis would allow us to truly identify whether ATZ aggregated in the ER possesses a phenotype that is not significantly different to its aggregation in the cytoplasm. In order to do this, both the secreted and non-secreted A1AT transgenic animals would have to be integrated and possess similar levels of expression. This would eliminate the variable of an increased expression level causing a greater degree of phenotypic response. If it is then confirmed that aggregation in the cytoplasm causes similar phenotypes to aggregation in the ER, we can test known mutations known to cause extracellular serpins to aggregate on the human intracellular serpins. Many serpins that have been research possess regions of highly conserved amino acid residues. Moreover, it is often seen that common disease mutations found on specific extracellular serpins occur in areas of conserved amino acids. By testing these mutations on intracellular serpins, we

can then identify whether the mutations that cause extracellular serpins to aggregate in the ER will also cause an intracellular serpin to aggregate in the cytoplasm. If this is then proven, similar phenotypic testing can be carried out.

Overall, it is clear that the introduction of intracellular ATM and ATZ produces similar phenotypic responses to the secreted variants of A1AT. With further investigation towards the affects of intracellular aggregation, we may develop a better understanding as to how the cell handles cytoplasmic aggregated proteins. And more importantly, this could lead to identifying the existence of intracellular serpinopathies that have yet to be discovered.

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Philip Jay Rosenstock

pjr5064@gmail.com
Cell Phone: (412) 225-7044

School Address:
203 East Fairmont Avenue, Apt. 319
State College, PA 16801

Home Address:
230 Sunridge Road
Pittsburgh, PA 15238

Education

The Pennsylvania State University University Park, PA 16802

- *Schreyer Honors College*
- Eberly College of Science, B.S. May 2011
- Major: Science, Life Science Option
- Minor: Film Studies

Honors/Awards

- Ruth E. Duffy Premedicine Endowment
- Schreyer Ambassador Travel Grant
- Phi Beta Kappa
- Dean's List every semester

Future Experience

Teach for America, Colorado Corps Fall 2011-Spring 2013

Secondary Mathematics Teacher

- Accepted to teach in an underprivileged school district of Colorado.
- A full-time teaching position with the goals of closing the state and national achievement gaps.

Experiences

UPMC Children's Hospital, Pittsburgh PA 15201 Summer 2009 and 2010

Neonatology Department (Dr. Gary Silverman, MD, PhD, Chief of UPMC Newborn Medicine)

- National Institute of Health funded research in *C. elegans* morphology, development, and physiological properties
- Analyzed phenotypic variations resulting from three common newborn disease-type mutations.
- Continued research with Hanna-Rose Research Group (PSU) from Fall 2009-Spring 2011
- Schreyer Honors College thesis drawn from this research

PSU Alpha Epsilon Delta, National Health Preprofessional Honor Society Fall 2007-Present

- President – Responsible for the overall function of the 250+ member club. 2010-2011
- Secretary – Published minutes, communicated regularly to club members, and co-chaired on the chapter's philanthropic committees. 2009-2010
- Selected twenty professional speakers to educated members in various aspects of all health related fields.
- Organized the PSU/MSU Blood Drive Challenge and The AED Public Health Fair.
- Participated in campus-wide volunteering and philanthropic events such as the Penn State IFC/Panhellenic Dance Marathon raising $9.56 million for pediatric cancer research.

UPMC Neonatal and OBGYN Shadowing Summer 2009 and 2010

- Observed several live births.
- Oversaw neonatal rounding in the Newborn Intensive Care Unit.
- Learned various aspects of pediatric care and emergency medicine.

PSU Nonotechnology Department (Dr. Ray Schaak) Fall 2008-Spring 2009

- Performed undergraduate research in nanotechnology.
- Analyzed results to make recommendation on future projects pertaining to gold nanorod synthesis and galvanic replacement reactions.

Penn State University Health Services Peer Interventionist Fall 2009-Spring 2010

- Selected to receive 30 hours of training in peer counseling through the Health Promotion and Wellness office.
- Counseled 50+ troubled peers in alcohol and marijuana intervention.

PSU Tropical Field Ecology Research (Dr. James Marden) December 2009-January 2010

- Designed and executed field research projects in rain forest, cloud forest, beach and mountain climates across Western Costa Rica.