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ELUCIDATION OF NOVEL DELAYED HATCHING PHENOTYPE OBSERVED IN *CAENORHABDITIS ELEGANS*

TENIOLA IDOWU
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Reviewed and approved* by the following:

Wendy Hanna-Rose, Ph.D.
Department Head, Biochemistry and Molecular Biology
Professor of Biochemistry and Molecular Biology
Thesis Supervisor

ABSTRACT

The connection between genetics and environmental conditions has become increasingly relevant to understanding disease conditions. Both genetics and environment can affect longevity and lifespan by decreasing the metabolic rate of an organism. *C. elegans* containing a knock-out mutation in the *nmrk-1* gene do not synthesize an enzyme known as nicotinamide riboside kinase, which is involved in the synthesis of NAD+. NAD+ is an important chemical reaction cofactor and metabolic signaling molecule. Importantly, higher levels of NAD+ have been linked to cell longevity and decreased aging. A combination of this genetic mutation and and environmental stressor results in a novel delayed hatching phenotype exhibited in eggs laid by *nmrk-1* mutants. In our case, the environmental stressor is UV-killed food, which the worms are raised on. In an effort to characterize and understand more about this phenotype, I worked to determine factors that can affect its strength. I was able to determine that the developmental stage at which *nmrk-1* mutants are exposed to UV-killed food increases the length of the delay. Also, using RNAi knockdowns I was able to investigate the role of the hexosamine pathway in the progression of this phenotype. These findings help to explore the connection of genetics and the environment, and to further understanding the role of NAD+ in *C. elegans* development.

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ACKNOWLEDGEMENTS

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Chapter 1

Introduction NAD+ Function and Biosynthesis

Nicotinamide adenine dinucleotide (NAD+) is a coenzyme that plays a role in many cellular processes that support proper metabolic function. It acts an electron carrier to catalyze many redox reactions, such as glycolysis, and without proper NAD+ levels many of these processes would be compromised (Johnson et al., 2018). NAD+ is an important metabolite that plays a role in many processes associated with aging and overall lifespan, and loss of NAD+ is associated with metabolic disorders and neurodegenerative disease (Johnson et al., 2018). Research using a variety of models that display decreasing NAD + levels have also supported that its reduction signals the start of senescence (Canto et al., 2015). Also, studies on NAD+ using the model organism *Caenorhabditis elegans* have shown that the reduction of NAD+ levels starts the development of many processes associated with aging (Canto et al., 2015). These findings emphasize the role NAD+ plays in metabolism and also highlights the possibilities of using it as a therapeutic target. *C. elegans* has been a very useful model to study NAD+ function and synthesis and they are capable of producing NAD+ using three pathways.

The *de novo*, salvage and riboside pathways are three forms of NAD+ synthesis found in *C. elegans*(Fig.1). In the *de novo* pathway, NAD+ is synthesized from the amino acid tryptophan. It closely mimics the process in humans except for one step that involves the conversion of quinolinic acid to nicotinic acid mononucleotide (McReynolds et al., 2017). Other organisms using this pathway have a quinolinic acid phosphoribosyltransferase (QPRTase) to catalyze this step, however, *C. elegans* was recently discovered to use the UMPS-1 enzyme in order complete this step and complete NAD+ synthesis (McReynolds et al., 2017). This

newfound use *of de novo* synthesis in *C. elegans* aids in maintaining homeostasis and proper reproduction in the worms.

In the salvage pathway, NAD+ is synthesized by recycling products of its degradation, such as nicotinamide (Sporty et al., 2009). Nicotinamide is then converted to nicotinic acid mononucleotide, which is then used to generate more NAD+ via the *de novo* pathway (Canto et al, 2015). The salvage pathway provides intermediates that are then used in *de novo* synthesis of NAD+, which shows how connected these pathways are. This connection is efficient and many mammals rely on the salvage pathway to maintain their NAD+ levels (Canto et al., 2015).

The riboside pathway uses nicotinic acid to synthesize NAD+ (de Figueiredo, 2011). Nicotinic acid is then phosphorylated by nicotinamide riboside kinase (NMRK-1) to make nicotinamide mononucleotide (NMN) (Belenky et al., 2007). The loss of *nmrk-1* results in a decrease in NAD+ levels and causes an extension of embryogenesis by delaying the hatching process of *C. elegans*. NMN then undergoes a reaction catalyzed by NMAT 1 and NMAT 2 to produce NAD+ (Denu, 2007).

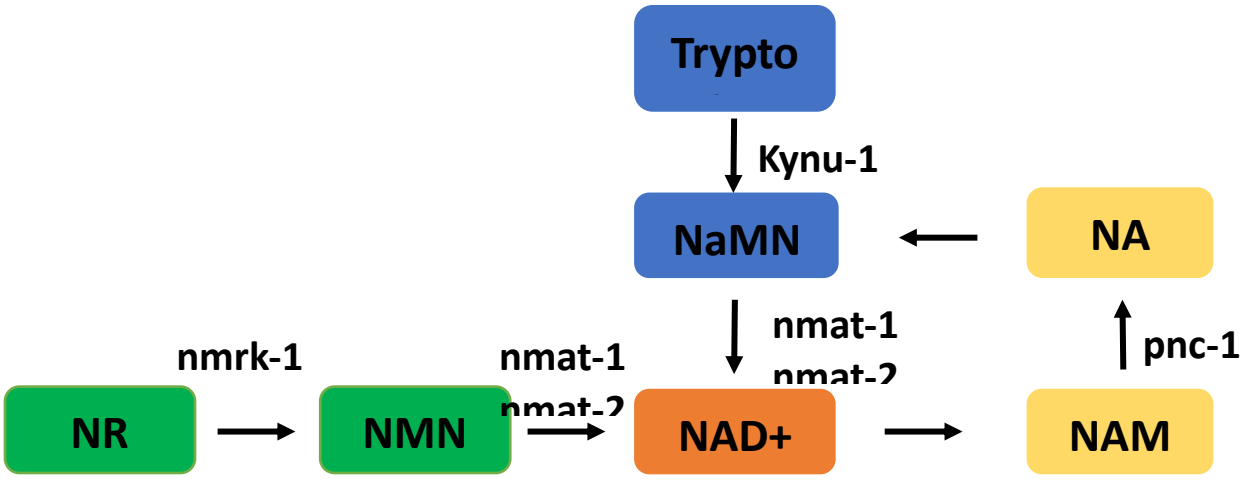


Figure 1. Pathways of NAD+ synthesis in *C. elegans*.
NAD+ can be produced by the riboside pathway (green) beginning with nicotinamide riboside. It can also be synthesized from scratch starting with tryptophan via the de novo pathway (blue). Degradation of NAD+ produces nicotinamide (NAM), which is then recycled to nicotinic acid mononucleotide (NaMN) using the salvage pathway (yellow). NaMN is then used to synthesize NAD+ using the de *novo* pathway.

Embryogenesis and Development of *C. elegans*

C. elegans undergo a 3.5-4-day life cycle beginning with the development of the embryo followed by four more stages known as the L1, L2, L3 and L4 stage (Byerly et al., 1976). However, the length of this life cycle is temperature dependent and is shorter in our lab. During embryogenesis, a single cell goes through proliferation where it undergoes hundreds of cell divisions (von Ehrenstein and Schierenberg, 1980). After this period, the cells near the surface of the embryo elongate and undergo specific divisions and differentiation that results in a three-fold shape representing the larval stage worm (von Ehrenstein and Schierenberg, 1980). The embryo begins to twitch as a result of increased muscle activity and the worm begins to move inside of the eggshell (Altun and Hall, 2020). The shape of the worm continues to elongate and takes on a slimmer shape. Once the shape is fully developed and the nervous system is matured, the worm undergoes hatching and is freed from the eggshell, which completes embryogenesis.

Development from this point is triggered by feeding (Ambros, 2020). After eating, *C. elegans* continue to undergo cell divisions 3 hour post-hatching. It will pass through 4 larval stages before reaching adulthood. During these larval stages, the worm grows larger and many of its body systems are developed. Specifically, during the L4 stage, the reproductive system and structures necessary for egg-laying are also developed (Altun and Hall, 2020). Approximately 48 hours post-hatch, the worm is now capable of laying its first set of eggs.

The *C. elegans* Eggshell

The eggshell of *C. elegans* is made up of six layers. The first layer is known as the outer vitelline layer, followed by the chitin layer and ending with the chondroitin proteoglycan (CPG)

layer (Olson et al., 2012). The vitelline layer coincides with the plasma membrane of the oocyte and begins to separate during fertilization (Stein and Golden, 2018). Not much is known about this layer, however, when it has been removed from the shell using sodium hypochlorite the embryo continues to develop normally (Stein and Golden, 2018). The chitin layer preserves the shape of the eggshell and provides mechanical support (Stein and Golden, 2018). Without this layer, the eggshell would not maintain its proper ovoid shape. The third layer used to be known as the lipid-rich layer, but recent work has shown that it is made up of proteoglycans (Olson et al., 2012). The final three layers consist of the extra-embryonic matrix, the permeability barrier and the peri-embryonic layer (Stein and Golden, 2018). The matrix helps to separate the outer eggshell from the plasma membrane of the oocyte (Stein and Golden, 2018). The permeability barrier helps to regulate the osmotic pressure of the shell and it is composed of lipids (Stein and Golden, 2018). The final layer sits tight below this barrier and provides extra protection of the embryo (Stein and Golden, 2018).

Currently, the process of hatching is relatively unknown for *C. elegans* and is better described for other parasitic worms (Barrett, 1976; Perry et al., 1991; Wharton, 1986). Right before hatching, *C. elegans* embryos start a process of where their pharynx begins pumping up until hatching, which is known as pharyngeal pumping (von Ehrenstein and Schierenberg, 1980; Sulston et al., 1983; Bird and Bird, 1991). Parasitic worms are known to release enzymes that digest the eggshell, so the belief is that *C. elegans* may use a similar process to properly hatch from the eggshell (Barrett, 1976; Perry et al., 1991; Wharton, 1986).

It takes at least 8 hours for wildtype eggs to hatch, however, hatching can be delayed and this is exhibited in *nmrk-1* mutants. When these mutants are raised on UV-killed OP50, hatching can be delayed for many days post egg-lay. The delay has been seen 4,8 and even 10 days post

egg-lay. My goal has been to further characterize this phenotype by determining factors that influence the length of the delay seen in this phenotype.

UV-Killed Food Sources and Oxidative Stress

C. elegans are raised on agar plates containing a strain of *E.coli* known as OP50. Often times when the worms are supplemented with metabolites, UV-killed OP50 is used to prevent bacterial metabolism from consuming these supplements (Brenner, 1974). When compared to wild-type worms kept on live OP50, worms raised on UV-killed food exhibited lower levels of NAD+, and NAD+ precursors (Wang, 2014). In addition to reduced NAD+ levels, these worms had increased levels of vitamin C and methionine sulfoxide, which are biomarkers of oxidative stress (Finkel and Holbrook, 2000). This suggests that UV-killed food is less nutritious due to reduced nutrients and can lead to oxidative stress in strains raised on it (Wang, 2014; Wang et al., 2015).

The Hexosamine Pathway

The hexosamine biosynthetic pathway is composed of four enzyme-mediated steps that help to produce uridine diphosphate N-acetlyglucosamine (UDP-GlcNAc). UDP-GlcNAc is an important substrate involved in protein glycosylation and it is typically found playing a structural role in cells (Konopka, 2012). This pathway begins with GFAT-1 and GFAT-2 which are the enzymes that help to convert fructose-6-phosphate from glycolysis into glucosamine-6-phosphate, which is the first substrate of this pathway (Fig.2). Through the three remaining steps, this pathway produces UPD-GlcNAc which undergoes a few more steps to produce chitin and chondroitin, which are components of the eggshell (Fig.2).

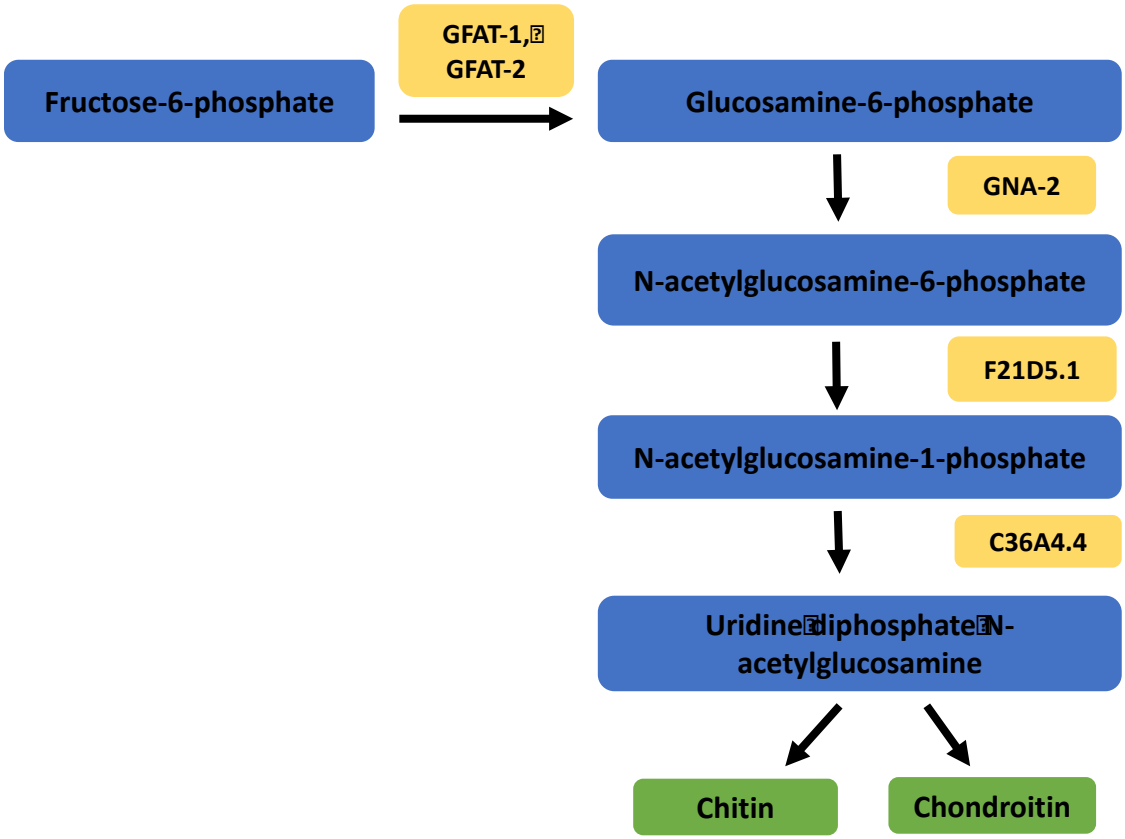


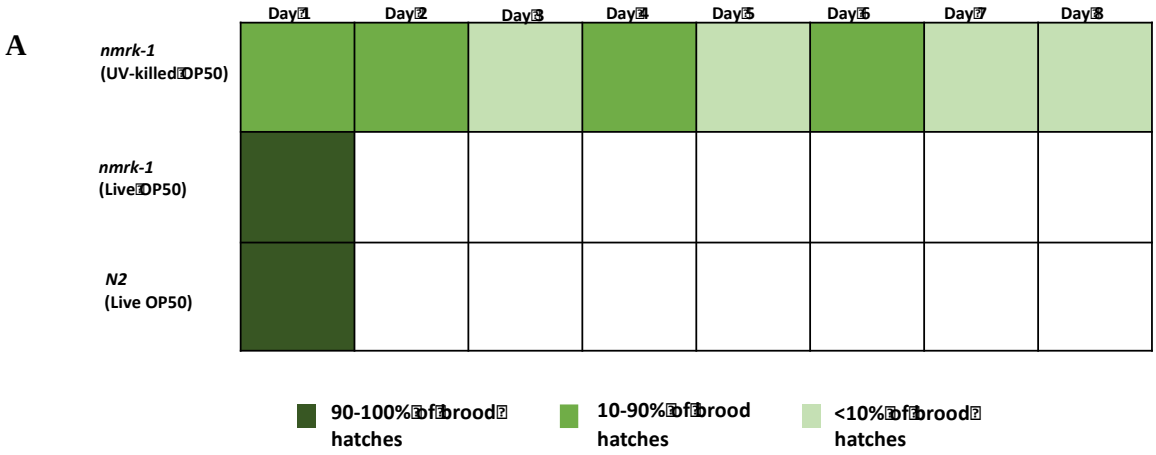
Figure 2. The hexosamine pathway.
The hexosamine pathway produces uridine diphosphate N-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc is further modified to produce chitin and chondroitin, which are both components of the *C. elegans* eggshell.

Chapter 2: Results

Environmental Stress and NMRK-1 Deficiency Results in Delayed Hatching Phenotype (DHP)

To determine what factors influence the delayed hatching phenotype seen in *nmrk-1* mutants, I first replicated the delayed hatching assay in order to see the characteristics of the phenotype. I compared wild-type N2 worms to *nmrk-1* mutants on dead-food environments. Adult-stage worms from each strain were raised on UV-killed OP50 and their eggs were scored for delayed-hatching. To score the eggs, I came in every 24 hours to count the number of eggs remaining on the plate and new hatchlings. Afterwards, I would remove any newly hatched worms. This occurred every day until all the eggs hatched. The eggs of the N2 worms grown on UV-killed food had 100% hatching 24 hours after they were laid (Fig.3). The eggs laid by *nmrk-1* mutants grown on live OP50 also exhibited 100% hatching after 24 hours post egg-lay. Delayed hatching was only seen in the eggs of *nmrk-1* worms kept on UV-killed OP50 (Fig.3). Delayed hatching was seen up to 8 days in these worms, with the majority of the brood hatching 48 hours post egg-lay (Fig.3). These results supported the fact that in order to have the delayed hatching phenotype, *C. elegans* must not have the NMRK-1 enzyme in addition to being put on UV-killed food. The UV-killed food acts as an environmental stressor in some way to result in this phenotype. In addition to the delayed hatching, eggs of *nmrk-1* worms that were kept on UV-killed food experience a high percent of lethality that is typically around 50% (Fig.3). These eggs never hatch and we are not sure yet what is causing the lethality associated with the phenotype. In this case, I was able to see delayed hatching up to 8 days post egg lay, however, other people who have used these mutants in the lab have seen delayed hatching up to 5 or 10 days. So, there

is a difference in the extent of delayed hatching seen in these worms, and I wanted to determine what factors affect the strength of this phenotype.



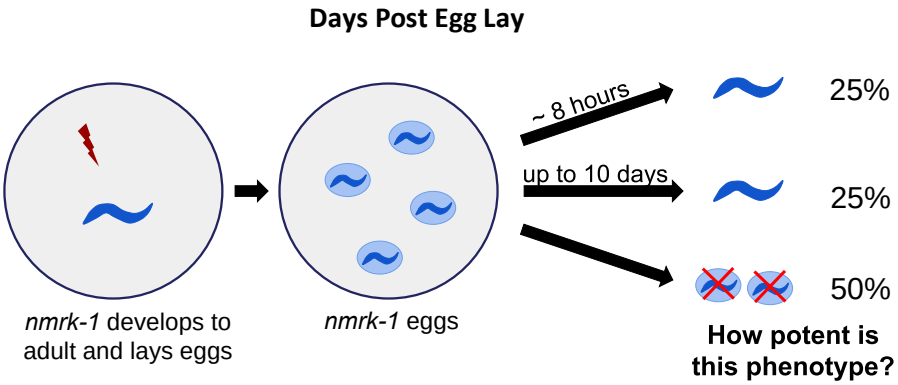
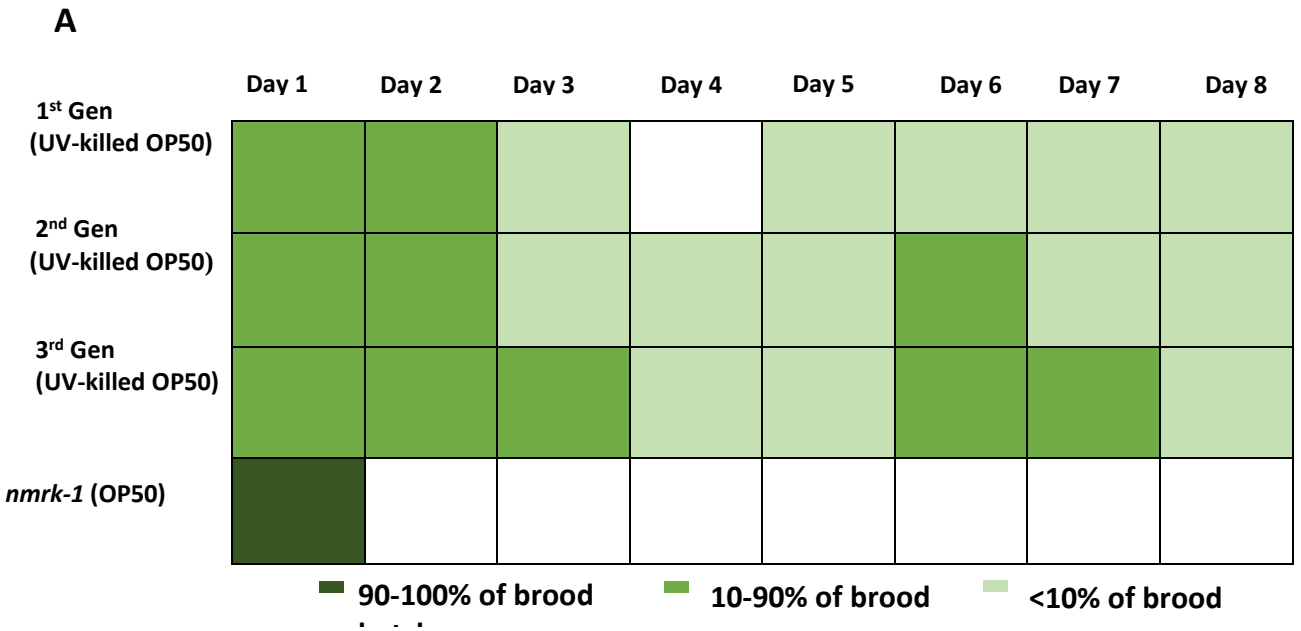


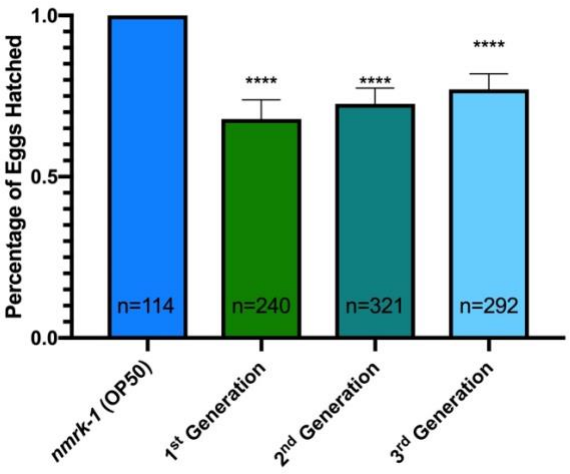
Figure 3. Delayed hatching is only exhibited by *nmrk-1* worms kept on UV-killed OP50. **(A)** Heat map showing N2 and *nmrk-1* mutants kept on live OP50 exhibit normal hatching with all of the eggs hatching 24 hours post egg-lay. *nmrk-1* worms kept on UV-killed OP50 experience delayed hatching up to 8 days post egg-lay. **(B)** Diagram showing that *nmrk-1* mutants plus UV-killed food results in the delayed hatching phenotype.

Robust DHP Is Observed Regardless of Number of Generations on Dead Food

To determine what factors influence this phenotype, I decided to see how the length of time *nmrk-1* mutants spend on dead food affects the phenotype. The worms are kept on dead food for different amounts of time before using them for experiments, so

time spent on dead food was a factor that changes between experiments. To decide whether the strength of the phenotype is a result of longer exposure to a dead food environment, I exposed *nmrk-1* mutants to a dead food environment for 1-3 generations. If there was a significant difference in the number of days post-egg lay that I saw hatching, the length of exposure would play a role in the strength of the phenotype. The progeny of the first, second and third generations of *nmrk-1* mutants kept on dead food were scored for DHP. The eggs were compared to the eggs of *nmrk-1* mutants kept on live food. As expected, the eggs of *nmrk-1* mutants kept on live food displayed normal hatching with all of the eggs hatching 24 hours post egg-lay (Fig.4). However, the eggs of the *nmrk-1* mutants kept on dead food for 1-3 generations all exhibited a strong delayed hatching phenotype, with hatching seen up to eight days post egg-lay. Despite the difference in generation time, there was no significant difference in the phenotype between the generations. Focusing on the lethality aspect of the phenotype, all three generations had approximately 30-40% lethality, with eggs from the first generation experiencing the highest lethality. The difference in lethality between the generations is not significant, but the phenotype is robust regardless of the generation time. These results revealed that the amount of time these worms spend on dead food is not a factor that results in the variability of this phenotype.





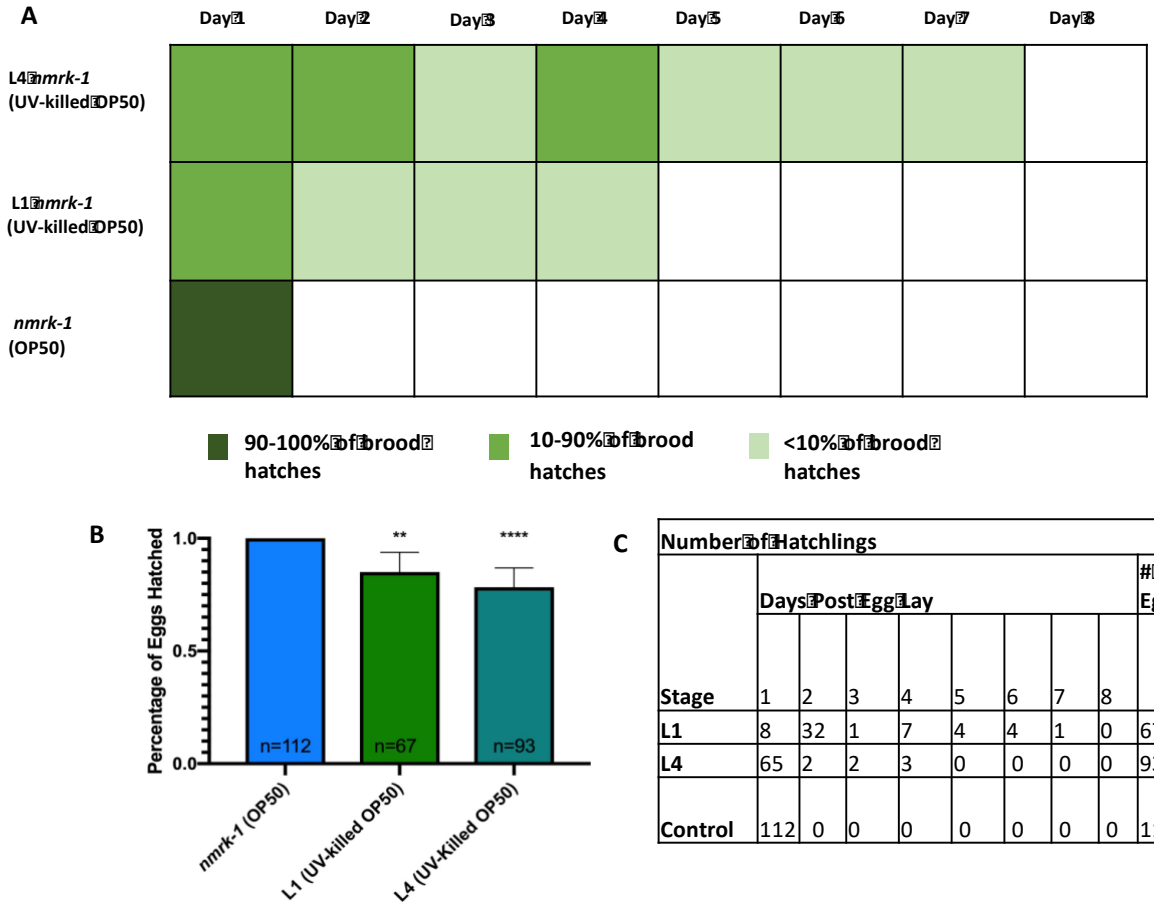
C

	Number of Hatchlings								
	Days Post Egg Lay								# of Eggs
Stage	1	2	3	4	5	6	7	8	
GT 1	125	16	5	0	6	5	6	1	240
GT 2	182	21	9	2	4	11	4	1	321
GT 3	139	23	15	10	6	20	12	2	292
Control	114	0	0	0	0	0	0	0	114

Figure 4. Delayed hatching phenotype is robust, despite the different generation times of *nmrk-1* mutants kept on UV-killed OP50.
(A) *nmrk-1* mutants kept on live OP50 exhibit normal hatching with all of the eggs hatching 24 hours post egg-lay. *nmrk-1* worms kept on UV-killed OP50 for 1-3 generations experience delayed hatching up to 8 days post egg-lay. **(B)** Significant difference in lethality seen in *nmrk-1* mutants on dead food for 1-3 generations versus *nmrk-1* kept on live OP50. ***=p<0.001 using ANOVA. **(C)** Table showing the exact number of eggs that hatched each day post egg-lay.

DHP is Stronger in *nmrk*-1 Worms Exposed to UV-killed OP50 at L1 stage

Another factor I wanted to test is the developmental stage at which the mutants are exposed to UV-killed OP50. To do so, I exposed the mutants to dead food at two different developmental stages, L1and L4. The L1 stage is immediately after the worm hatches from its eggshell. The L4 stage is approximately 28 hours post egg-lay and it is the point at which the reproductive system of the worm is fully developed (Altun and Hall, 2020). Once the mutants reached these respective stages, I moved them to dead food plates and scored their offspring for delayed hatching. Mutants exposed to dead food since the earlier L1 stage exhibited delayed hatching up to 7 days post egg-lay (Fig.5). Those exposed at the L4 stage only saw delayed hatching up to 4 days post egg-lay (Fig.5). Both groups exhibited approximately 20% lethality, which is significant when compared to the *nmrk-1* mutants on live food control (Fig.5). The difference in the delay between the worms exposed at each stage suggests that exposing *nmrk-1* mutants at an earlier developmental stage could potentially strengthen the delayed hatching phenotype.



C

Number of Hatchlings									
	Days Post Egg Lay								# of Eggs
Stage	1	2	3	4	5	6	7	8	
L1	8	32	1	7	4	4	1	0	67
L4	65	2	2	3	0	0	0	0	93
Control	112	0	0	0	0	0	0	0	112

Figure 5. Delayed hatching phenotype is stronger in *nmrk-1* exposed to UV-killed food at the earlier L1 stage.

(A) *nmrk-1* mutants kept on live OP50 exhibit normal hatching with all of the eggs hatching 24 hours post egg-lay. *nmrk-1* worms kept on UV-killed OP50 since the L1 stage experience delayed hatching up to 7 days post egg-lay, whereas worms exposed since the L4 stage experience it up to 4 days. **(B)** Significant difference in lethality seen in *nmrk-1* mutants on dead food since L1 and L4 stage versus *nmrk-1* kept on live OP50. ***=p<0.001, **=p<0.01 using ANOVA. **(C)** Table showing the exact number of eggs that hatched each day post egg-lay.

The Hexosmaine Pathway Produces a Low Percent of Lethality in *gfat-1* Knockdowns

In addition to determining what external factors are potentially affecting this phenotype, I also looked into potential metabolic pathways that could also be playing a role. Thanks to another member of the delayed hatching team, Elizabeth Eberly, we were able to obtain images of the eggshells of *nmrk-1* and wildtype *C. elegans*. When comparing these images, we were able to see that the CPG layer of the *nmrk-1* eggshells were thicker than the wildtype layer. As a result, a new hypothesis came into play. The new idea was that the thicker eggshell is making it harder for the mutants to actually hatch. One pathway that makes up the components of the CPG layer is the hexosamine pathway, so the next step of my project was to determine if this pathway is necessary for delayed hatching. To determine this, I used RNAi knockdowns targeting the hexosamine pathway to see if the delayed hatching phenotype is rescued. Specifically, I used RNAi to knockdown the expression of *gfat-1* and *gna-2*, which are enzymes involved in catalyzing the first two steps in this pathway. In addition to these knockdowns, I also used an empty vector control. To make sure the RNAi actually worked, I also knockdown the expression of *unc-22*, which is known to cause a visible twitching phenotype in the worms. Once I was able to confirm the twitching, I knew the RNAi was working properly. I placed L1 worms onto plates spotted with RNAi then scored their offspring for delayed hatching. As expected, the empty vector control exhibited delayed hatching up to 4 days post egg-lay (Fig.6). Mutants containing the *gfat-1* knockdown experienced delayed hatching up to 4 days post egg-lay and *gna-2* knockdowns did not exhibit any delayed hatching (Fig.6). Focusing on lethality, the eggs of the *gfat-1* knockdowns had a low lethality of 1.4%, whereas eggs of the *gna-2* knockdown had almost 100% lethality (Fig.6). Although delayed hatching was still exhibited in worms with a *gfat-1* knockdown, the low lethality suggests a rescue of the phenotype. Since there was a very

high lethality seen in the *gna-2* knockdowns it is not possible to really test if the phenotype was rescued or not.

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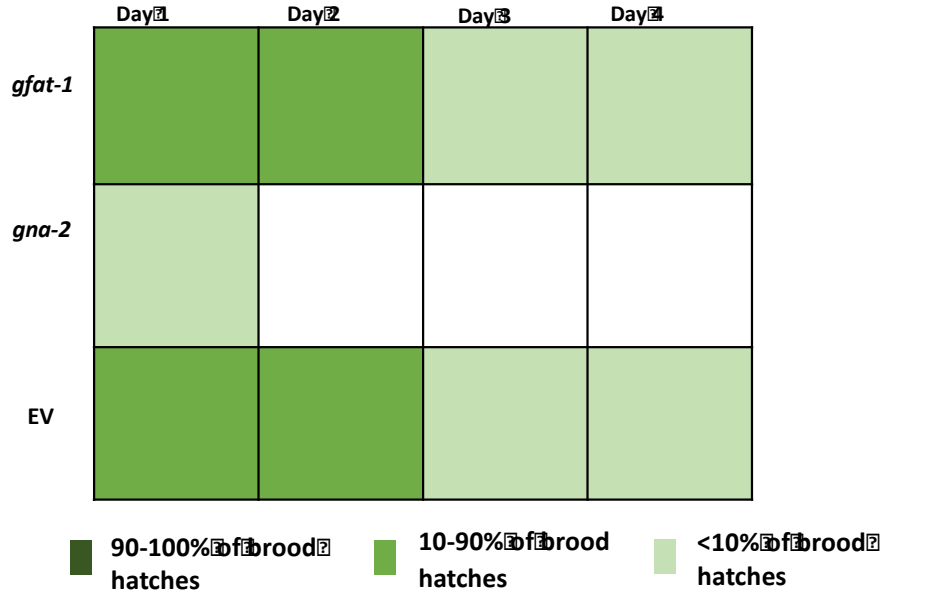

B

Number of Hatchlings							
	Days Post Egg Lay				Total Number of Eggs	No Hatch	%Lethality
RNAi Clone	1	2	3	4			
gfat-1	436	162	25	13	645	9	1.4
gna-2	1	0	0	0	542	541	99.8
EV	252	175	48	21	558	62	11.1

Figure 6. The hexosmaine pathway produces a low percent of lethality in *gfat-1* knockdowns. **(A)** Heat map showing the hatching pattern of the empty vector control and RNAi knockdowns targeting the hexosamine pathway. **(B)** Table showing the exact number of eggs that hatched each day post egg-lay and the percent lethality of each group.

Chapter 3: Discussion

In this study, I was able to further characterize the delayed hatching phenotype seen in *nmrk-1* worms. First, I was able to confirm the characteristics of this phenotype and was able to verify that it requires *nmrk-1* worms to be raised on UV-killed OP50. Seeing that the delay varied up to different days, I hypothesized that some other factors must be at play to affect the strength of the phenotype. As a result, I tested to see how the length of time these mutants spend on UV-killed OP50 affects the phenotype. By comparing the phenotype on mutants that remained on food for 1-3 generations I was able to show that despite the length of time, there is still a robust delayed hatching phenotype seen in the worms. In fact, regardless of each generation, the delayed hatching was consistently seen up to 8 days post egg-lay, which suggests that this is not a factor that is capable of influencing the phenotype.

The next factor I decided to test was the developmental stage at which the *nmrk-1* worms are exposed to UV-killed OP50. Comparing the delayed hatching strength of both groups, the worms exposed at the earlier L1 stage had experienced delayed hatching three days longer than the worms exposed since the L4 stage. The phenotype was stronger in the worms exposed since the L1 stage which suggests that the stage at which *nmrk-1* worms are exposed to a dead food environment does affect the phenotype. Exactly what molecular mechanism is causing this difference is unknown, but it could have something to do with what is occurring in *C. elegans* at each point in their development. At the L1 stage the worms are freshly hatched and many of their bodily systems have not fully matured by this point. At the L4 stage the development of the reproductive system is completed. So, the dead food environment could have a stronger effect at the developing stage of the reproductive system, which may explain the difference. However, more developmental stages should be compared before this can be concluded.

Based on the eggshell images, I was motivated to look into the molecular basis of this phenotype and focused on the hexosamine pathway. Using RNAi to inhibit the first step of the pathway did slightly rescue the delayed hatching phenotype because *gfat-1* knockdowns had a very low lethality percentage. Since *nmrk-1* mutants typically have a lethality of 50%, the loss of *gfat-1* expression was able to help lessen that effect of the phenotype. However, inhibiting the second step resulted in only one egg hatching and a high lethality percentage of 99%. Since only one egg hatched, it is hard to conclude whether or not the phenotype was rescued. This would be a worthwhile experiment to repeat while making sure to target other steps of the hexosamine pathway as well. Although I was not able to determine the role the hexosamine pathway plays in the *nmrk-1* delayed hatching phenotype, its potential role in this phenotype has not been ruled out.

Chapter 4: Implications

The results of this study have further implicated the connection between exposure to a UV-killed OP50 environment to NAD+ metabolism. Seeing that earlier exposure at the L1 stage in *nmrk-1* worms increases the strength of the phenotype leads me to want to understand how this difference is possibly changing the molecular mechanisms underlying the phenotype. Understanding the role these external factors play is important because it can help us understand how it influences the functions of our metabolic pathways. Understanding more about how manipulations to NAD+ producing pathways influence the development of an organism can allow us to potentially find new ways of targeting NAD+ as a therapeutic.

The results of these experiments have been able to determine a potential new role of the hexosamine pathway in the development of the delayed hatching phenotype. Seeing that the CPG layer is thicker in the eggshells of *nmrk-1* mutants, suggests there is increased flux into the hexosamine pathway. The knockdown of *nmrk-1* results in a decrease in NAD+ biosynthesis, which potentially results in an increased flux in the hexosamine pathway. This results in a higher production of chondroitin, which creates a thicker CPG layer in *nmrk-1* eggshells. As a result, it is more difficult for the embryos to exit the shell (Fig.7). Being able to determine more about the connections between NAD+ synthesis and its role in the hexosmaine pathway can help us to further characterize the delayed hatching phenotype. As mentioned earlier, NAD+ plays a role in many processes associated with aging, so learning more about its role in *C. elegans* development and physiology can help us understand ways to improve human health span later on down the line.

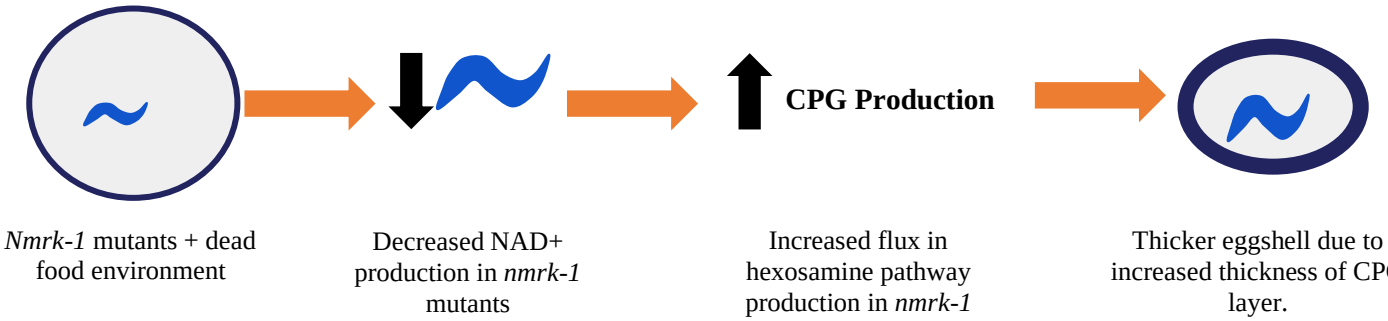

Figure 7. Potential model of delayed hatching phenotype as a result of increased flux in hexosamine pathway.
When *nmrk-1* mutants are combined onto dead food, it results in decreased NAD+ synthesis. This results in oxidative stress that causes increased flux into the hexosamine pathway. As a result, there is an increased production of CPG that produces a thicker eggshell in the progeny of the mutants.

Chapter 5: Materials and Methods

Nematode Strains and Maintenance

N2 and *nmrk-1 C. elegans* strains were maintained on NGM plates at 20˚C (Brenner, 1974). All plates were seeded with live OP50 and allowed to dry for a 24-hour period before being used to raise worms. UV-killed OP50 plates were prepared by exposing plates to UV radiation for 600 seconds using a GS Gene Linker UV Chamber (BioRad, Hercules, CA).

Delayed Hatching Analysis

Worms were maintained at mixed stages on live OP50. 5-8 gravid adults were transferred to live or UV-killed OP50 and remained on the plates to lay eggs for four hours. After this time, the worms were removed and the eggs on the plates were counted. Every 24-hours I would count the remaining number of eggs and hatchlings on the plates and removed the new hatchlings until there were no more eggs remaining. I recorded the percentage of eggs that hatched each day and the total percentage of the eggs hatched out of the brood.

RNA interference

The *gfat-1* and *gna-2* RNAi clones were from the *C. elegans* RNAi Library (Source BioScience, Nottingham, UK). Liquid cultures were shaken overnight at 37˚C and IPTG was added to 0.4 mM the next day. These cultures were then shaken at 37˚C for two hours and spotted on RNAi plates. The plates were allowed to dry for 24-hours and the bacteria were killed by treatment in a GS Gene Linker UV Chamber (BioRad, Hercules, CA) for 600 seconds. L1 *nmrk-1* animals were transferred to RNAi plates, and their progeny were allowed to reach adulthood. The eggs laid by these adults were then scored for delayed hatching.

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ACADEMIC VITA

Teniola Idowu

9873 Good Luck Rd Apt.1, Lanham, MD • 240-374-2203 • teniolaidowu98@gmail.com

Education

The Pennsylvania State University University Park, PA

- Major: Microbiology, B.S.
- Minor: Sociology
- Anticipated Graduation: December 2019

Academy of Health Sciences at Prince George's Community College Largo, MD

- General Studies, Associate's Degree

Awards

Gates Millennium Scholarship *April 2016-Present*

- One of only one-thousand accepted students from the 53,000 applications to the final class of Gates Millennium Scholars
- Receive a full tuition and room & board fellowship valued at $220,000
- Participate in networking and professional development workshops

Penn State Millennium Scholars Program *March 2016-Present*

- A merit-based scholarship program designed to prepare students for the pursuit of doctoral degrees in science, technology, engineering and mathematics (STEM) disciplines.

Research Experience

***Student Researcher*, The Pennsylvania State University** *December 2016-December 2019*

PI: Dr. Wendy Hanna-Rose

- Conduct on campus research focused on understanding the role of NAD+ metabolism on developmental phenotypes in *C. elegans.*
- Will complete an honors thesis of work by December 2019.

***Research Intern,* New York University** *June – August 2019*

PI: Dr. Ana Rodriguez

- Conducted research as part of NYU'S Summer Undergraduate Research Program in the Microbiology department.
- Investigated the *Plasmodium falciparum* factors initiating the loss of blood brain barrier integrity associated with cerebral malaria.

***Research Intern,* Genentech (Immunology Department)** *May – August 2018*

PI: Dr. Mark Wilson

- Conducted research focused on epithelial cell biology.
- Investigated the mechanisms of epithelial cell death in airway inflammation.

***Research Intern*, The Jackson Laboratory** *June-August 2017*

PI: Dr. Jennifer Trowbridge

- Conducted research in a lab focused on the epigenetics controlling hematopoiesis and leukemia.
- Investigated the role of the methyltransferase, KMT5A, on the development of myeloid cells during hematopoiesis.
- Trained in various mice biomethod techniques such as peripheral bleeds, IP injections, cervical dislocation, ear notching, etc.

Leadership

***Programs Chair*, National Organization for the Professional Advancement of Black Chemists and Chemical Engineers (NOBCChE)** *August 2017-December 2019*

- Plan and organize events to encourage underrepresented groups of students to remain in STEM, and promote a safe and fun community of minority students in STEM.

***Advanced Facilitation Trainer*, World in Conversation** *May 2019- December 2019*

- Collaborate weekly with a team of four colleagues to deliberate on design and delivery of over 80 hours of workshop and class instruction for advanced facilitation team in applied sociology per semester.

***Member*, Afrique Fusion** *August 2017-May 2018*

- Performed historical and modern African dances at different events on campus in an effort to showcase African culture to the Penn State community.

***Dialogue Assistant II*, World in Conversation** *May 2017-May 2019*

- Observe and critique discussions on race and ethical relations that help students understand new perspectives on issues, while learning more about their own identities and personal beliefs.

Poster and Oral Presentations

- **Idowu T**., Zuniga M., Gomes C., Rodriguez A. 2019." *Plasmodium falciparum* Factors that Induce Brain Endothelium Disruptions in Malaria." Poster presentation at the Annual Biomedical Research Conference for Minority Students (ABRCMS).
- **Idowu T**., Zuniga M., Gomes C., Rodriguez A. 2019." *Plasmodium falciparum* Factors that Induce Brain Endothelium Disruptions in Malaria." Oral Presentation at the Leadership Alliance National Symposium (LANS).
- **Idowu T**., Hanna-Rose W. 2019. "Elucidation of Novel Delayed Hatching Phenotype Observed in *Caenorhabditis elegans*" Poster presentation at Penn State's Undergraduate Research Exhibition.
- **Idowu T**., Hanna-Rose W. 2018. "Elucidation of Novel Delayed Hatching Phenotype Observed in *Caenorhabditis elegans*" Poster presentation at the Annual Biomedical Research Conference for Minority Students (ABRCMS).
- **Idowu T**., Teng G., Wilson M. 2018. "Cell Death in Severe Asthma" Oral Presentation at Genentech Immunology Department Meeting.
- **Idowu T.,** Huso V., Hanna-Rose W. 2018. "Elucidation of Novel Delayed Hatching Phenotype Observed in *Caenorhabditis elegans*" Poster presentation at Penn State's Undergraduate Research Exhibition.
- **Idowu T**., Borikar S., Trowbridge J. 2017." Identification of Non-Histone Protein Targets of KMT5A in Hematopoietic Cells" Oral presentation at The Jackson Lab Summer Research Symposium.

Conferences

- Annual Biomedical Research Conference for Minority Students (ABRCMS), Anaheim, California November 2019
- Leadership Alliance National Symposium (LANS), Hartford, Connecticut July 2019
- Annual Biomedical Research Conference for Minority Students (ABRCMS), Indianapolis, Indiana November 2018

Additional Awards

- 2019 ABRCMS Poster Presentation Award
- Joseph A. Miller Scholarship in Science Award
- Dean's List: Fall 2017, Spring 2019