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EXPLORING THE REGULATION OF THE NUCLEAR HORMONE RECEPTOR NHR-114

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EXPLORING THE REGULATION OF THE NUCLEAR HORMONE RECEPTOR NHR-114

by

GUFRAN TAHA

Presented to the Faculty of the Honors College of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

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HONORS BACHELOR OF SCIENCE IN BIOLOGY

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April 13, 2020

ABSTRACT

EXPLORING THE REGULATION OF THE NUCLEAR HORMONE RECEPTOR NHR-114

Gufran Taha, B.S. Biology

The University of Texas at Arlington, 2020

Faculty Mentor: Mark Pellegrino

A significant threat in the medical field today is the rise of antibiotic resistance, which challenges physicians and can severely limit patient survival outcomes. Discovering new means of protecting the host against infection are therefore needed. Using the model organism *Caenorhabditis elegans*, we previously discovered that we could increase host survival during infection with the opportunistic pathogen *Pseudomonas aeruginosa* by simple supplementation with the branched chain amino acid leucine. Using genetics, we found that the nuclear hormone receptor NHR-114 mediated this pro-survival benefit of leucine supplementation. In an effort to understand the mechanism of NHR-114 function, we explored its spatial regulation and localization using GFP reporters. Using this approach, we find that while transcription of *nhr-114* is robust, NHR-114 protein levels are inherently unstable. We subsequently used a forward genetics approach to identify the

cause of NHR-114 instability. Unfortunately, our initial screen did not yield any promising candidates.

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CHAPTER 1

INTRODUCTION

1.1 Discovery of Leucine as a Pro-Survival Amino Acid During Infection

Mitochondria are complex organelles that mediate a variety of essential functions including the generation of cellular energy. Dysfunction to mitochondria must efficiently be mitigated to avoid cellular and organismal decline. The mitochondrial unfolded protein response (UPRmt) is one pathway that is activated to restore homeostasis (Shpilka & Haynes, 2017). In *C. elegans,* this stress response is regulated by the bZIP stress activated transcription factor ATFS-1 (Nargund et al. 2012). ATFS-1 contains both a mitochondrial targeting sequence (MTS) and a nuclear localization signal (NLS). Under healthy conditions ATFS-1 is imported into mitochondria, but during stress the efficiency of this import pathway is reduced. Consequently, ATFS-1 accumulates in the cytoplasm and subsequently enters to nucleus to transcribe genes that restore mitochondrial homeostasis (Shpilka & Haynes, 2017).

The UPRmt also has a role in the regulation of innate immunity during infection with pathogens that target mitochondrial function including the opportunistic pathogen *Pseudomonas aeruginosa* (Pellegrino et al. 2014). However, the UPRmt is also repressed with chronic *P. aeruginosa* infection (Deng et al., 2018). We previously found that *P. aeruginosa* FadE2 mediates this repression of the UPRmt. FadE2 is an acyl CoA dehydrogenase involved in the catabolism of the branched chain amino acids leucine and valine. The working model is that FadE2 mediates the harmful effects of infection by

reducing metabolism and the UPRmt by sequestering leucine catabolites from the host. Consistent with this model, leucine or valine supplementation can overcome the activities of FadE2 and prolong the survival of the host.

1.2 NHR-114 Mediates the Protective Effects of Leucine Supplementation

We wished to understand the mechanism of protection during infection via leucine supplementation. We had previously performed transcriptomics analysis of *C. elegans* exposed to wild-type *P. aeruginosa* and a *P. aeruginosa* mutant for the FadE2 gene. Among the genes that were differentially expressed was the nuclear hormone receptor *nhr-114*. Interestingly, while loss of NHR-114 had no effect on wild-type *C. elegans* survival, it completely suppressed the extended survival afforded with leucine supplementation, one of the branched chain amino acids metabolized by FadE2. The objective of the current study was to gain a further understanding of NHR-114 regulation.

CHAPTER 2

METHODOLOGY

2.1 Extrachromosomal Array Generation

Extrachromosomal arrays are created when foreign DNA is introduced into *C. elegans* via microinjection transformation. Here, DNA consisting of P*nhr-114*::GFP or P*nhr-114*::NHR-114::GFP was injected into the distal arm of the gonad (Evans, 2006). The DNA is then incorporated into developing oocytes which eventually give rise to transgenic embryonic progeny. A needle was filled with 1µl of the DNA injection mix and the tip was centered on a microscope's field using the 5X objective. A drop of oil was placed on the injection pad. Worms grown on OP50 NGM were carefully transferred from a plate, using a clean pick, to the oil droplet. The worms were neatly arranged all facing the same direction. The injection pad was then transferred to the microscope stage for the worms to be injected. Injection of DNA material should be made with the dorsal side of the worm at a 15-45 angle to the needle.

2.2 CRISPR/Cas9 Genome Editing

In nature bacteria have a genome editing system that allows them to take portions of invading virus DNA and store it in CRISPR arrays in case of later invasion by similar viruses. These CRISPR arrays give the bacteria the ability to produce viral RNA to target the virus. CRISPR/Cas9 genome editing allows for the editing of host DNA sequence including the introduction of exogenous encoding genes such as green fluorescent protein (GFP) (Reis, Hornblower, Robb, & Tzertzinis, 2014). We used CRISPR/Cas9 technology

to insert the GFP DNA sequence at the end of the *nhr-114* open reading frame, removing the endogenous stop codon of *nhr-114* (Calarco & Friedland, 2015). This was performed so that we could have a transgenic *C. elegans* animal that was not overexpressing multiple copies of the NHR-114::GFP transgene which occurs when DNA exists as an extrachromosomal array.

2.3 EMS Forward Genetics Screen

P*nhr-114*::NHR-114::GFP transgenic *C. elegans* that were created using CRISPR/Cas9 were used for a forward genetics screen using ethyl methanesulfonate (EMS) as a mutagen. In this screen, the guanine nucleotide of the respective DNA sequence is exchanged for the abnormal O^6-ethylguanine creating various point mutations within the genome (Kutscher & Shaham, 2014). The homozygous F2 generation was compared alongside the wild-type and were screened for mutant animals that expressed NHR-114::GFP at a higher level.

Stage L4 worms were harvested and lysed to form a suspension. A solution of 2ml 2X EMS solution to 2 ml of worm suspension was placed in a fume hood and allowed to sit for four hours before the EMS was removed. Gravid P0 adults were isolated to 200 culture plates before harvesting, these worms were subsequently lysed to obtain F1 embryos which gave rise to the homozygous F2 generation (Jorgensen & Mango, 2002). Mutagenized F2 worms were screened for the novel GFP expression. Our control was the wild-type P*nhr-114*::NHR-114::GFP *C. elegans.*

CHAPTER 3

RESULTS

3.1 Expression of P*nhr-114*::GFP and P*nhr-114*::NHR-114::GFP

Transcriptional and translational GFP reporters were used to examine the expression and localization of NHR-114, respectively. Interestingly, we found that the transcriptional GFP reporter of *nhr-114*, which comprised the *nhr-114* promoter fused to the coding sequence of GFP (P*nhr-114*::GFP), was robustly expressed throughout the animal (Figure 3.1). However, the P*nhr-114*::NHR-114::GFP transgene, which includes the *nhr-114* coding sequence showed no detectable expression. This suggests that the NHR-114 protein is highly unstable or is targeted for degradation through an unknown mechanism. We therefore wished to use an unbiased genetics screen to identify mutants that restored the expression of P*nhr-114*::NHR-114::GFP.

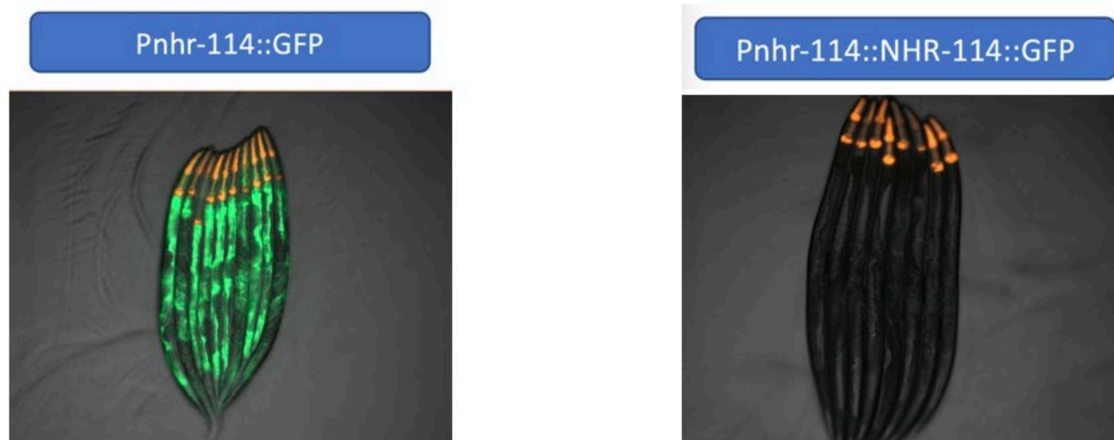

Figure 3.1: Expression of P*nhr-114*::GFP or P*nhr-114*::NHR-114::GFP in wild-type *C. elegans*. Note that the red fluorescence is from P*myo-2*::RFP which is used as a transformation marker.

3.2 CRISPR/Cas9 Genome Editing of P*nhr-114*::NHR-114::GFP

Through the use of CRISPR/Cas9 genomic editing (Figure 3.2), we were able to create an otherwise wild-type *C. elegans* that expressed GFP within the endogenous locus of *nhr-114*. Taking our gene of interest, the GFP reporter was inserted directly adjacent to *nhr-114* coding sequence (the stop codon of *nhr-114* was removed in the process as to not perturb the *nhr-114* reading frame). As expected, we observed no expression of GFP fluorescence much like the P*nhr-114*::NHR-114::GFP that was expressed as an extrachromosomal array.

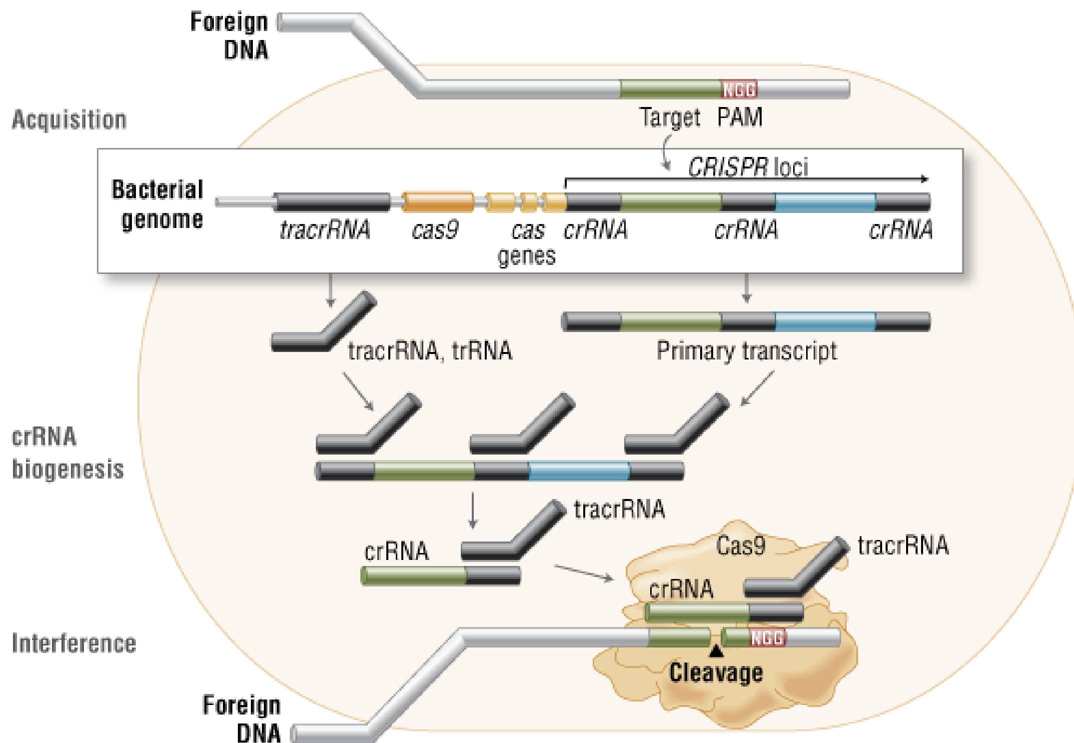

Figure 3.2: CRISPR/Cas9. Adapted from, "CRISPR/Cas9 & Targeted Genome Editing: New Era in Molecular Biology." (Reis, Hornblower, Robb, & Tzertzinis, 2014)

3.3 Forward Genetics Screen

Using the P*nhr-114*::NHR-114::GFP transgenic animal created by CRISPR/Cas9, we screened for mutants that could increase the expression of the GFP using EMS-mediated forward genetics (Figure 3.3). The F2 generation of mutagenized animals were screened but unfortunately, we did not observe any animals that expressed GFP.

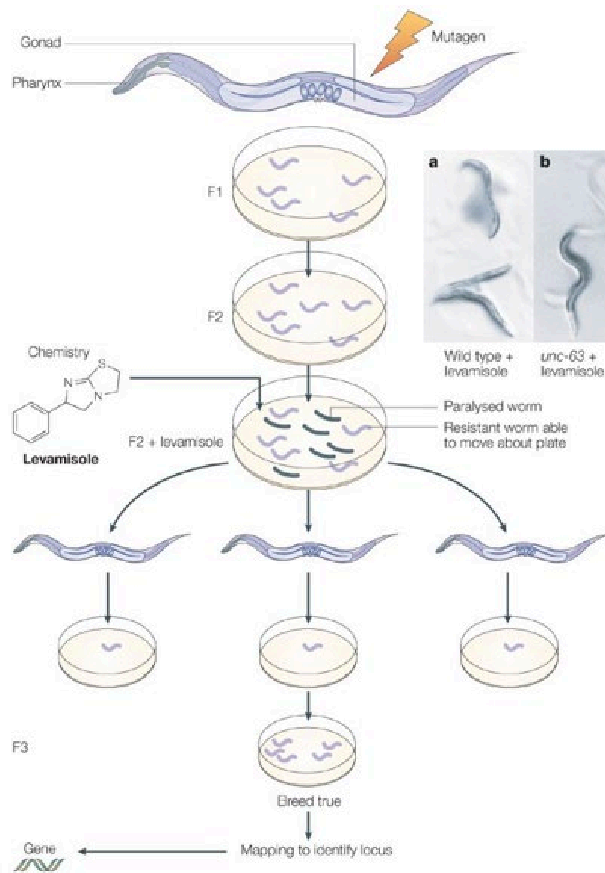

Figure 3.3: Forward Genetics Scheme. Adapted from, "Chemistry-to-Gene Screens in *Caenorhabditis elegans*." (Jones, Buckingham, & Sattelle, 2005)

CHAPTER 4

DISCUSSION

The goal of this research was to study the regulation of the nuclear hormone receptor NHR-114. Using transcriptional and translational GFP reporters, we were able to conclude that NHR-114 is highly expressed at the transcriptional level but is likely turned over at the protein level. Unfortunately, despite our efforts, we were unable to uncover the mechanism behind the rapid turnover of NHR-114. One possibility is that NHR-114 protein levels need to maintained at a very low level in order to promote survival. In this case, even small increases may reduce *C. elegans* viability. This might explain why we couldn't detect any mutants that expressed the GFP since increased NHR-114 levels might have killed the animal. Another possibility is that the mechanism used to turn over NHR-114 itself is essential for worm viability, in which case we would still not observe any animal expressing the GFP reporter due to increased lethality. Lastly, the issue may be technical. For example, the EMS mutagenesis itself may not have been efficient. In line with this possibility, we did not observe many other "classical" mutant phenotypes that should have arisen from mutations in other genes. For example, uncoordinated and dumpy phenotypes which create slow moving or small worms, respectively, are commonly observed. However, neither type of mutant was observed in our screen, which suggests that the EMS mutagenesis may not have been efficient.

4.1 Future Research

We plan to repeat this screen using fresh EMS reagent in order to ensure efficient mutagenesis of *C. elegans*. The absence of other mutant phenotypes (e.g. uncoordinated, dumpy) might be explained by an old batch of EMS reagent and therefore this would resolve this issue.

We will also repeat the screen but this time focusing on the isolation of temperature-sensitive mutants. These types of mutants give rise to phenotypes only when raised at elevated temperatures. In this manner, we could still culture our mutant at lower incubation temperatures. This should resolve the issue of mutations in essential genes that might affect our ability to recover mutants in our screen. If successful, we will then backcross the mutant with wild-type *C. elegans* to remove unlinked mutations, and then prepare genomic DNA to be used for whole genome sequencing (Moresco, Li, & Beutler, 2013). We will then determine which genes have mutations in their coding sequence that result in changes in the amino acid sequence which might affect its function. Rescue experiments will then be performed wherein the wild-type DNA of each gene is reintroduced into our mutant animal and examined for its ability to restore wild-type levels of P*nhr-114*::NHR-114::GFP, thus indicating that it is the causative gene of interest.

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BIOGRAPHICAL INFORMATION

Gufran Hassan Taha will be graduating in the Spring of 2020 with an Honors Bachelor of Science in Biology. During her time at the University of Texas at Arlington (UTA), she focused on building her portfolio as a competitive medical school applicant making sure to maintain a 3.8+ GPA and gaining experience in the world of medicine. Through her dedication, she was able to sustain her Maverick Academic Scholarship making classes like Organic Chemistry and Human Physiology GPA boosters. She will also graduate Magna Cum Laude, which afforded her a position on the Dean's List for the Spring 2020, Spring 2018, and Fall 2018 semesters.

Ms. Taha was involved in a multitude of extracurricular experiences some affiliated with the university and others not. Serving first as a member and later as the Education Chair and Secretary of the Muslim Student Association at UTA, the MSA served as her entrance into the world of religious studies and the power of diversity within an organization. She completed and presented a poster presentation and research paper while researching under Dr. Matthew Walsh as part of an Undergraduate Research Experience. Within the same year she was introduced to the clinical side of her degree. As an Emergency Medical Scribe, Ms. Taha documented under several physicians including Dr. Carla Cash and Dr. Mohammed Hamzeh at THR Arlington Methodist Hospital.

Gufran will be applying to medical school this cycle with hopes of continuing her education in healthcare. Her intention is to is to use the knowledge she will gain to treat citizens of all communities but especially that of her mother country Sudan.