University of Nevada, Reno

Towards an understanding of alterations in the expression profile of the ADL olfactory neuron of *Caenorhabditis elegans* as a function of feeding state

A thesis submitted in partial fulfillment
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by

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Abstract

Olfaction is adjusted in many organisms in response to changes feeding state. The proposed study intends to describe a method of investigating the molecular mechanisms involved in changes in olfactory perception as a function of feeding-state in the model organism *Caenorhabditis elegans*, a system characterized by an expansive understanding of important genetic and neural networks. Alterations in gene expression in the olfactory neuron due to feeding state will be characterized using molecular techniques and next-generation sequencing. Such an understanding of these mechanisms could be utilized to prevent infection of human hosts by parasitic nematodes and other parasites as well.

Acknowledgements

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Introduction

Olfaction is an important sensory perception possessed by many organisms. As a chemosensation, olfaction is one of the older sensory modalities exhibited by metazoans, and subsequently a wide variety of different biological processes and functions are linked to olfaction (i.e. pheromones and mate choice, determination of estrus cycles, construction of social hierarchies, and use in predation). Perhaps the most classical and adaptive usage of olfaction is that of finding food: predators smell prey, foragers sniff out possible meals, and the odor of a given food allows for discrimination as to whether it is good to eat or rotten. On the molecular level, this relationship between "smelling" and "eating" is realized by different neuromodulatory mechanisms that alter olfactory perception and feeding behavior. Some of these changes take place via long-term processes involving genetic manipulation of olfactory neurons, in which specific genes encoding for olfactory receptors are either up-or down-regulated due to changes in food intake. Evolutionarily, an adaptive strategy would consist of a heightened sense of smell during absence of food (starvation), as an increased ability to detect odors will lead to an increased probability of finding a meal. Similarly, periods of food abundance (well-fed) corresponds to a decreased olfactory perception. The molecular realization of these olfactory perceptual changes consists of alterations in gene expression in response to feeding state. An example of such genetic manipulation is observed in the mosquito species *Anopheles gambiae*, the vector for the protozoan malaria parasite *Plasmodium falciparum* (Hallem *et al.*, 2004). Female *A. gambiae* mosquitos possess an olfactory receptor AgOr1, which is able to detect 4-methylphenol, an odorant component found in human sweat. Expression of this olfactory receptor is down regulated in response to a blood meal— that is, fewer receptors for the human sweat odorant are present after the mosquito has fed. This particular example of olfaction in

mosquitos is of great interest, not only for the elegant demonstrations of interactions between environmental, sensory inputs and resultant genetic changes and expression effects, but also for essential clinical reasons. If an understanding of how different parasites sense their hosts using chemosensory perception apparatuses could be achieved, then possible prevention techniques for a huge number of infectious diseases (i.e. malaria, dengue fever, dysentery) could in turn be realized. Knowledge of such genetic manipulations to sensory perception could have a wide variety of applications, particularly in certain medical considerations. For example, a familiarity of the protein pathways utilized to express newly synthesized olfactory receptors could allow for development of a drug which could precisely knockout necessary molecular intermediaries needed for novel odorant detection, thereby preventing olfactory detection in a given species. While the mosquito *Anopheles gambiae* is such a species with obvious clinical implications, many other organisms with epidemiological significance are less understood in terms of alteration of olfaction as a result of feeding state.

Nematodes, which make up the phylum Nematoda, are a hugely significant infectious parasite for humans all around the world. Incredibly, nematode infections of the gastrointestinal tract affect half of the world population (Stepek *et al.*, 2006). In developing countries, specifically, helminths, a polyphyletic group which includes nematodes (roundworms) as well as platyhelminths (flatworms), are the most prevalent infectious agents of humans: it is predicted that about one third of the poorest populations of areas such as Sub-Saharan Africa, Asia, and the Americas are infected with helminthes (Hotez *et al.*, 2008). Helminthic infections primarily tend to infect children, resulting in stunted growth and impaired cognition (Crompton and Nesheim, 2002), and are also regularly coendemic with both malaria and HIV; specific diseases caused by nematode infections include ascariasis, trichinosis, and schistosomiasis. Despite these obviously

very high medical impacts, very few anti-helminthic drugs exist today, and study of helminthic diseases receives less than 1% of global research dollars (Hotez, 2013). Thus, an understanding of olfaction in nematodes could potentially have very wide reaching pharmaceutical and subsequently epidemiological consequences for the most at-risk populations on the planet.

Of the thousands of different kinds of nematodes, none are as well understood as *Caenorhabditis elegans*. As a model organism, *C. elegans* is tremendously valuable, being characterized not only by a completely sequenced genome, but also a thorough tracking of every cell lineage present from fertilization, an extremely short generation time, an abundance of behavioral phenotypes, powerful molecular and genetic tools for experimentation, and a completely mapped nervous system. This last characteristic of *C. elegans* is of particular interest to neuroscientists, as an understanding of how 302 neurons connect to one another (as outlined by White, Thomson and Brenner, 1986) allows for an exploration into neural circuits and how neurons respond to signaling from other types of cells. Since the establishment of *C. elegans* as a powerful and tractable model organism, a wide variety of different methods have been developed that allow for analysis on several different levels of biological organization (molecular, genetic, transcriptional, cellular, neural, and organismal). One such technique (described in further detail below) is utilized in this thesis project to analyze RNA transcripts being produced from a single cell, the ADL neuron, one in a set of amphid neurons utilized in olfactory sensation of a variety of chemical stimuli (de Bono *et al*., 2002).

This thesis will focus on developing a technique to analyze how feeding state alters olfaction in the free-living nematode *Caenorhabditis elegans*, a model organism used for its expansive set of genetic and molecular tools. By using integration techniques, the poly-A binding protein transgene will be inserted into the *C. elegans* genome downstream of the ADL

cell-specific promoter *sre-1*. Subsequent immunoprecipitation and mRNA sequencing will allow for analysis of the transcription profile of a single olfactory neuron pair in *C. elegans*, as outlined in Figure 1. Analyzing the alteration in the transcription profiles can lead to clues as to what kinds of genes alter their expression by feeding state, and how such alterations occur. This research has pharmaceutical implications for prevention of helminthic parasitic infections of humans in developing countries around the world.

Literature Review

Alteration of olfactory receptor expression in *A. gambiae* following a blood meal is a clear example of the relationship between feeding state and olfactory perception. This demonstration closely models the goal of this particular thesis project: to observe the changes in olfaction as a result of feeding state in *C. elegans*. The olfactory system of *C. elegans* has been shown to play a role in many different nematode behaviors and life processes, such as locomotion, egg laying, and fat storage (Sawin, Ranganathan, and Horvitz, 2000; Waggoner *et al.*, 2000; Fujiwara, Sengupta, and McIntire, 2002), so an understanding of the effects feeding state has on olfaction carries important implications for comprehending the integrated whole of *C. elegans* biology. Other model systems have been observed in regards to how olfaction and feeding state are interrelated. For example, in zebrafish (species name *Danio rerio*), expression of different olfactory receptors is affected at least partially by the timing in the development of the organism (Barth, Dugas, and Ngai, 1997). The genes for different odorant receptors are grouped closely together on the chromosome, within a region of 100 kilo-basepairs: the multiplicity of these different olfactory genes is thought to have arisen through tandem gene duplication (Barth, Dugas, and Ngai, 1997). Most importantly, Barth, Dugas, and Ngai found that gene regulation for certain olfactory receptors occurs independently in distinct groups of olfactory neurons. That is, the regulation of genes encoding for olfactory receptors is not coordinated across olfactory neurons. If this model holds for all animals, then results generated by this thesis describing the ADL neuron in *C. elegans* may not translate over to the other amphid olfactory neurons in the *C. elegans* nervous system (such as ASH, ADK, ASI, etc.; see White *et al*., 1986). Research in fruit flies (*Drosophila melanogaster*), another popular model organism, reveals how different organisms have different olfactory receptor

organization and receptor specificity. *Drosophila* has two families of odorant receptor genes: one consisting of 61 "selectively expressed receptors" and *Or83b*, a broadly expressed olfactory receptor gene, and research has shown that *Or83b* affects expression of the other 61 olfactory receptor genes, as a higher order genetic regulator of detection of different kinds of odorants (Larsson *et al*., 2004). The prior two examples demonstrate the kind of information that can potentially be gathered from an analysis of *C. elegans* olfaction: in effecting feeding state, a great deal about olfactory regulation can be determined using transcriptional analysis.

Other studies have focused in on the study of olfaction to observe the relationship between olfactory perception and feeding state. Besides *Anopheles gambiae* investigated by Hallem *et al*. (as described above), other blood-sucking parasites have also been studied in regards to gustatory-olfactory interactions. In Reisenman *et al*., 2013, the blood-sucking insect *Rhodnius prolixus* was tested for preference of odorant chemicals as a function of being starved or non-starved. The odorants tested, including α-pinene, limonene, nonanal, and yeast derivatives, were examples of both host-odorants (odors originating to potential hosts for *Rhodinus prolixus*) and non-host odorants. Starved insects were found to be attracted to the host-odorants and repulsed by the non-host odorants, whereas non-starved insects showed no preference to host versus non-host odorants. These results support the findings in Hallem *et al.*, 2008: olfactory preference and detectability of host odors increases for starved parasites. The molecular mechanisms underlying feeding-dependent olfactory changes in *Rhodinus prolixus* are unknown; however, in *Drosophila*, insulin and neuropeptide F are known to modulate olfactory receptor sensitivity (Root *et al*., 2011), and dopamine (an important and widespread neurotransmitter) is known to modulate sucrose acceptance (Marella, Mann, and Scott, 2012). Given the differences in complexity between insects and helminths, different kinds of

neuromodulatory mechanisms may be observed: the techniques described in this project delineate a means to go about finding both variances and similarities in olfactory modulation between these two broad classes of animals.

While this project aims to describe more thoroughly the olfactory system of *C. elegans,* a fair amount of research has already been performed on olfactory perception in nematodes and helminths more generally, parasitic and free-living. For parasitic species, carbon dioxide is an attractive odorant as it usually indicates the presence of a potential host (most hosts engage in respiratory processes). For instance, in the three species of parasitic nematode *Anclyostoma caninum, Strongloides stercoralis,* and *Haemonchus controtus*, an activity response was discovered when the infective third-stage larvae of these different species were exposed to carbon dioxide gas at the concentration of that found in human exhalation (Sciacca *et al*., 2002). The larvae of these species were found to be attracted to the CO_2, a possible evolutionarily advantageous sensation method. In contrast to these findings, it has been found in free-living, non-parasitic nematodes, such as *C. elegans*, that while low concentrations of CO_2 are attractive, higher concentrations tend to have a repulsive effect: exposure of CO_2 to mobile *C. elegans* induces a halting of forward movement and an initiation of reversal (Hallem *et al*., 2008). Furthermore, it was found that starvation affects sensitivity to carbon dioxide in *C. elegans*, along with other molecular factors such as insulin and transforming growth factor β (TGF β). Thus, feeding state has inherent correlations to olfaction even for non-parasitic nematodes. This modulation in *C. elegans* detection of carbon dioxide has been found to be dependent on the BAG neuron, a chemosensory neuron (Hallem *et al*., 2011). Furthermore, while parasitic nematodes with ablated BAG olfactory neurons were not able to detect or chemotax towards higher carbon dioxide levels, detection of other volatile cues given off by different arthropod

hosts was still possible. This retaining of host detection despite loss of the BAG neuron demonstrates that CO_2 is not the only cue involved in host detection for parasitic nematodes, and that BAG is not the only olfactory neuron of importance for finding potential hosts. Piali Sengupta demonstrates in his review paper "The belly rules the nose: feeding state-dependent modulation of peripheral chemosensory responses" the effect of several different biological molecules on different olfactory neurons in *C. elegans*, such as ASH and AWC (Sengupta, 2013). For instance, while dopamine is known to decrease the ASH-mediated avoidance response to octanol in *C. elegans*, serotonin has been shown to increase this response. Thus, different neurotransmitter molecules acting on the same neuron within *C. elegans* may elicit different behavioral responses.

While the prior examples demonstrate the effect of external cues (i.e. ligands, hormones, and neurotransmitters) on the sensory response of olfactory neurons, the goal of this project is to understand the internal genetic manipulations occurring in olfactory neurons, and how such manipulations effect gene expression. This focus on expression is taken because prior research has demonstrated that regulation of chemoreceptor genes contributes to changes in several different molecular pathways within *C. elegans*, as well as different nematode behavioral phenotypes. Research by van der Linden *et al*. describes the interactions between *C. elegans* genes *kin-29*, a salt-inducible kinase, and *egl-4*, a cyclic GMP-dependent protein kinase, in altering chemoreceptor gene expression and subsequently behavioral phenotypes dependent on sensory detection (van der Linden *et al*., 2008). By using single-cell transcriptional analysis, this project will bring into the fold other genes that are affected by feeding state changes. Both chemoreceptor genes and other interacting genes will most likely experience expression changes as a response to starvation. For this project, this expression analysis will be focused on one pair

of olfactory cells in *C. elegans*, the chemosensory ADL neuron pair.

The ADL neuron pair consists of two amphid neurons which act as nociceptive (or pain-sensing) chemoreceptors exposed to the external environment, as modeled in Figure 2. The focus on the ADL amphid neurons, as opposed to other chemosensory neurons within *C. elegans*, is due to research by Chao *et al*. that has demonstrated that ADL modulates olfactory responses in other proximal neurons: specifically, the time it took for the ASH amphid neuron to respond to the presence of the chemorepellant octanol was differentially modulated by the ADL neuron depending upon whether the nematode was in the presence of food or off of the food plate for 10 minutes (Chao *et al*., 2004). This disparity in different food conditions indicates that feeding state is somehow effecting the neural mechanisms of the ADL neuron. ADL has also been shown to work with other amphid neurons (such as ASH and AIB) to elicit avoidance behaviors (generally characterized by motility assays). The ADL neuron is known to respond to a variety of different olfactory signals, including pheromones, as well as heavy metals: for example, research by Sambongi *et al.* demonstrated how both copper and cadmium ions in the environment of *C. elegans* generate an avoidance response, results indicating the nociceptive chemosensory nature of ADL (Sambongi *et al*., 1999) Additionally, research by van der Linden *et al*. has demonstrated that expression of *srh-234*, a candidate chemoreceptor gene which encodes for a class H serpentine receptor, is downregulated in the ADL neuron when *kin-29* is knocked out (van der Linden *et al*., 2008). This result suggests that the ADL neuron is effecting the olfactory perceptions of nematodes. These studies demonstrating the role ADL plays in sensory modulation makes it an excellent cell for studying changes in olfaction in response to feeding state. ADL can be used to model how the presence of food and olfaction interact, and how this interaction is realized on a molecular level.

Methodology

In order to analyze transcriptional changes in a single neuron, a transgenic technique (outlined in Takayama *et al.*, 2009) will be utilized (Figure 1). In this technique, animals are created that express the poly-A binding protein (*PAB-1*), which binds to the poly-A tails of mRNA transcripts. Fused to this protein are FLAG epitope sites, polypeptide regions that have a high affinity to anti-FLAG antibodies. The transgenic PAB-1 protein will bind to mRNAs expressed in the ADL neuron, which in turn will be pulled down using anti-FLAG antibodies to isolate mRNA transcripts using immunoprecipitation. The transgene called *3xFLAG::PAB-1* will be expressed solely in ADL neurons by using the *sre-1* cell-specific promoter.

This project, which aimed to build the vector construct for integration into a line of transgenic nematodes, was the first step in a larger endeavor to fully characterize alterations in gene expression in the ADL neuron in response to feeding state. In order to obtain transgenic worms with ADL-specific expression of *3xFLAG::PAB-1*, several steps must be taken. First, a plasmid containing the transgene as well as other necessary components was constructed. The elements making up this plasmid include *3xFLAG::PAB-1*, the *sre-1* promoter upstream of the gene of interest in order to achieve ADL-specific expression, and an ampicillin resistance gene, used for production of this plasmid in bacterial colonies. This vector construct and its components are shown in Figure 3.

The process of obtaining the plasmid construct described above can be explained in fairly straightforward molecular biological terms. The construct that was created in this project has two DNA components, the insert and the vector, both of which existed in different plasmids already present within the lab. The insert for the plasmid *3xFLAG::PAB-1* is the gene of interest, the transgene that is to be expressed in a line of *C. elegans* in order to analyze transcriptional

changes as an effect of feeding state. The vector component of the construct contains *sre-1*, the cell-specific promoter which specifies expression of *3xFLAG::PAB-1* to the ADL neuron. In order to create the construct described in this project, both the *3xFLAG::PAB-1* insert and the *sre-1* vector had to be isolated and successfully ligated together. The separation of the insert and vector components was achieved using restriction enzymes, molecules which cut at precise locations (palindromic sites) within DNA strands, and gel electrophoresis, a molecular technique which uses electric fields to separate DNA strands according to size. Once both the vector and the insert components of the construct were isolated, they were mixed together in a reaction with DNA ligase, an enzyme that fixes strands of DNA together. The ligation product was then used in a transformation reaction, a procedure used to insert plasmid vectors into a host organism, usually a bacterial host. In this project, a line of *Escherichia coli* cells termed "JM 109" cells were used for transformation: this particular line of cells was used due to the ease of transformation and distinctively high transformation efficiencies. During the transformation reaction, cells are exposed to DNA generated in the ligation reaction and are then allowed to develop (or grow) in what is typically called the "log phase" of bacterial asexual reproduction (Zwietering *et al*., 1990). These bacteria are then plated onto an agar plate containing ampicillin, an antibiotic. The presence of this antibiotic acts as a selection pressure for transformed bacteria: because the plasmid construct contains an ampicillin resistance gene, only *E. coli* that have been successfully transformed with this construct will survive in the presence of the antibiotic. *E. coli* are grown up overnight and selected for sequencing: plasmid DNA is isolated from bacterial colonies and sent to the Nevada Genomics Center, which uses automated Sanger sequencing to determine the nucleotide sequence of DNA samples.

Sequencing results were then analyzed to see if the necessary vector components were

present in the constructed plasmid. To do this, a search in the returned sequence was made for the known sequences of both the *sre-1* cell specific promoter and the *3xFlag PAB* insert gene. A positive result was indicated by the presence of these two components adjacent to one another in the sequence (see Figure 3), as this represents a proper ligation reaction and the vector construct of interest.

Discussion of results

The plasmid construct containing the *3xFlag PAB* insert downstream of the *sre-1* cell-specific promoter is still currently in the process of being isolated. Thus far, sequencing results have indicated the presence of vectors from which the *sre-1* promoter and the *3xFlag PAB* insert were sourced, rather than the ligation of these two source components together to form a novel construct. This, in turn, indicates a failure to isolate ligation reaction products from the original reactants. Several measures were made during the course of this project to ensure that reaction conditions for all of the steps made in constructing the vector plasmid were ideal and run to completion: for instance, restriction digest reactions were run over night in order to guarantee complete digestion of DNA substrate, and optimal concentrations for ligation reactions components were calculated and utilized.

Different cloning techniques exist which may have yielded different, more successful results in attempting to obtain the vector plasmid. For instance, a TOPO cloning technique would have allowed for isolation of the *3xFlag PAB* gene from the source plasmid (which was identified as plasmid 237), thus decreasing the likelihood of resultant bacterial colonies following the transformation procedure to contain source plasmid, rather than novel plasmid ("The technology behind TOPO® Cloning," 2014). The addition of cloning into a TOPO vector would have taken more time, but would have also ensured a more promising end result.

As stated above, construction of the vector plasmid is the first step in a larger experiment which plans to observe in its entirety the expression profile of the ADL neuron and how it changes in response to changes in feeding state. Upon completion, the constructed plasmid outlined above will be integrated into the genome of *C. elegans*, in order to create a stable transgenic line of organisms. The integration of this extra-chromosomal array into the genome is

necessary to insure for the presence of an inheritable gene encoding for *3xFLAG::PAB-1*. In order to determine that transformation procedures have gone successfully, the plasmid construct will be co-injected with the selectable marker *unc-122*p*::rfp*, which encodes for red fluorescent protein. Integration into the genome will be achieved by subjecting *C. elegans* nematodes to UV irradiation (a technique outlined in Jiu *et al*., 2009). Parental transgenic individuals (P0) will be allowed to reproduce F1 progeny. Amongst the F1, individuals expressing the specific *rfp* marker will be selected for cloning. The progeny of the F1, F2, will be screened in order to ensure complete inheritance of the *3xFLAG::PAB-1* transgene. To determine that the function of the ADL neuron has not been compromised in the transformation procedure, the olfactory abilities of the transgenic nematodes will be tested using a standard chemotaxis avoidance assay. In such an assay, worms are placed on a plate that has volatile repellant odorants present, and the movement away from the area containing the repellants is used as a signal representing proper ADL functioning.

Conclusion

Upon successful construction of vector plasmid, this research will go on to uncover alterations in ADL functioning. Results from this research will demonstrate the first observations of single-cell transcription profiling comparing gene expression changes in response to feeding state. While the method utilized in this project is simultaneously specific and extremely powerful in and of itself, it is also valuable insofar as the results from this project will promote an abundance of new research questions regarding *C. elegans* biology. Observations into what genes are effected by feeding state in the olfactory ADL neuron can be followed up by studies uncovering what pathways these genes are involved in, or how different expression levels of these multiple genes are interrelated. Finally, an understanding of these mechanisms has implications for clinical research that withholds the possibility of pharmaceutically preventing the infection of the most at-risk human populations in the world by some of the most prevalent and destructive parasites.

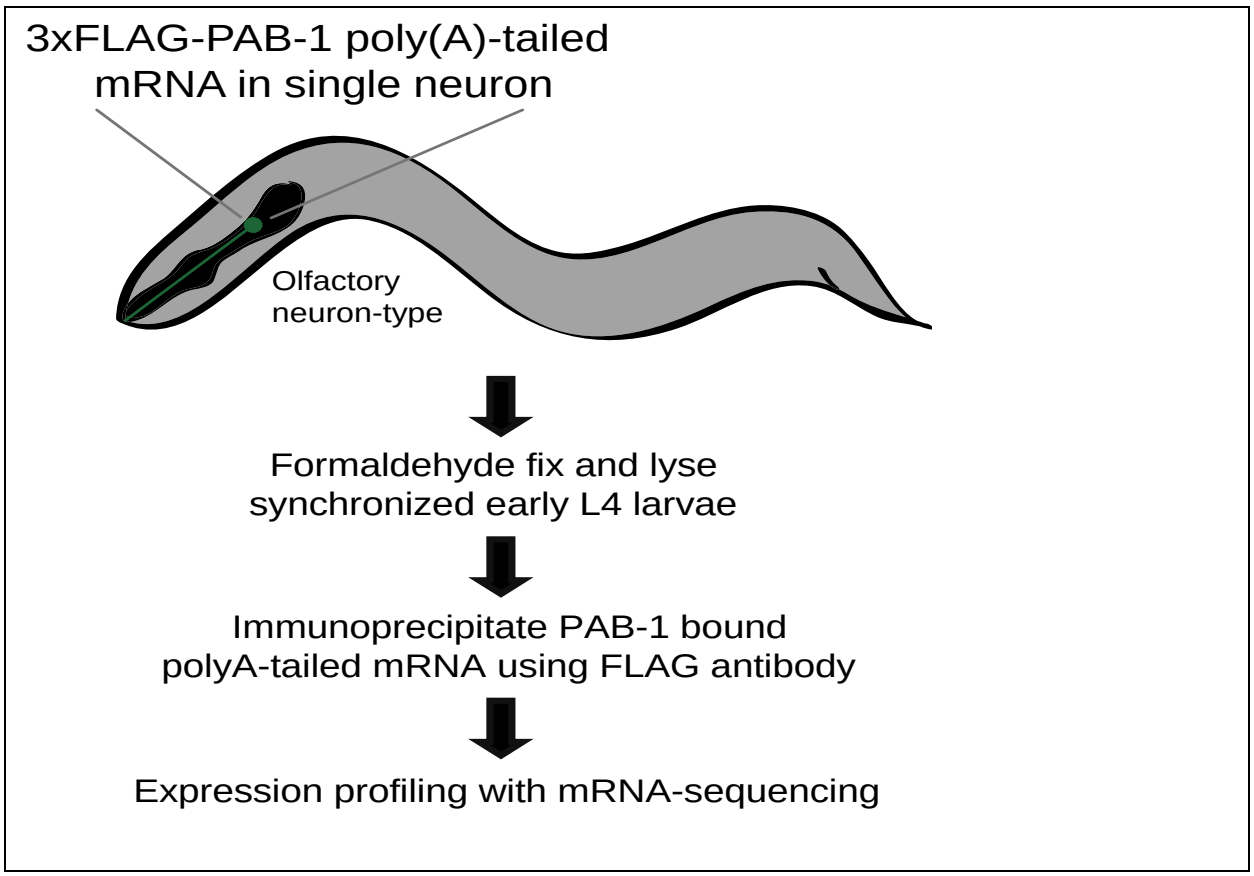

Figure 1, **Schematic describing single cell transcriptional analysis technique in *Caenorhabditis* elegans.** mRNA tagging approach to isolate RNA from transgenic animals carrying *3xFLAG::pab-1* specifically expressed in ADL neurons. ADL-specific expression is achieved by using the cell specific promoter *sre-1*. Immunoprecipitation isolates the poly-A binding protein amongst the thousands of other proteins present within the organism.

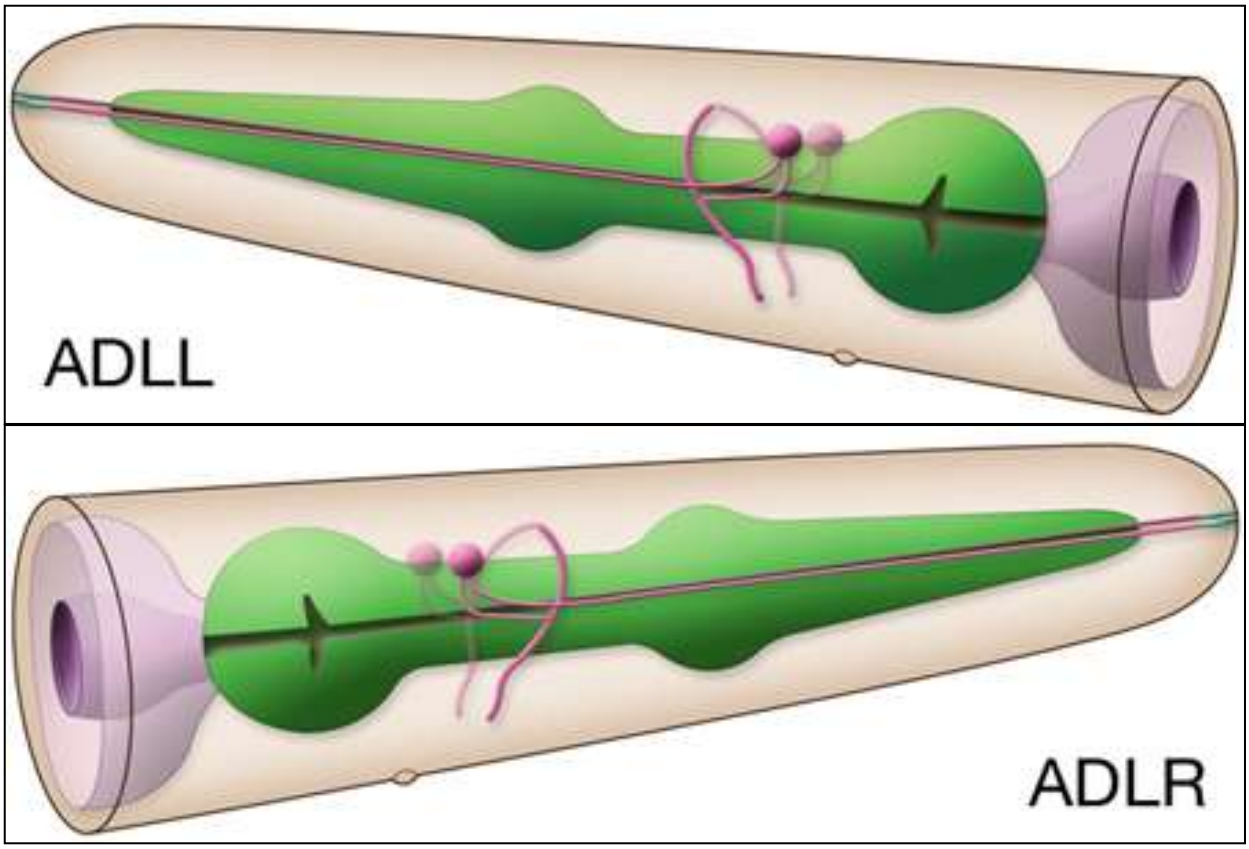

Figure 2, Model of the left and right ADL amphid neurons in the head of *Caenorhabditis elegans*. The left ADL neuron is displayed on top, while the right ADL neuron is displayed on bottom. The ADL neurons belong to a family of neurons called amphid neurons, which are generally categorized as the largest chemosensory organs of *C. elegans*. The neurons are shown in purple, and are seen to project within the amphid channel, allowing for exposure to the external environment ("WormAtlas," 2014).

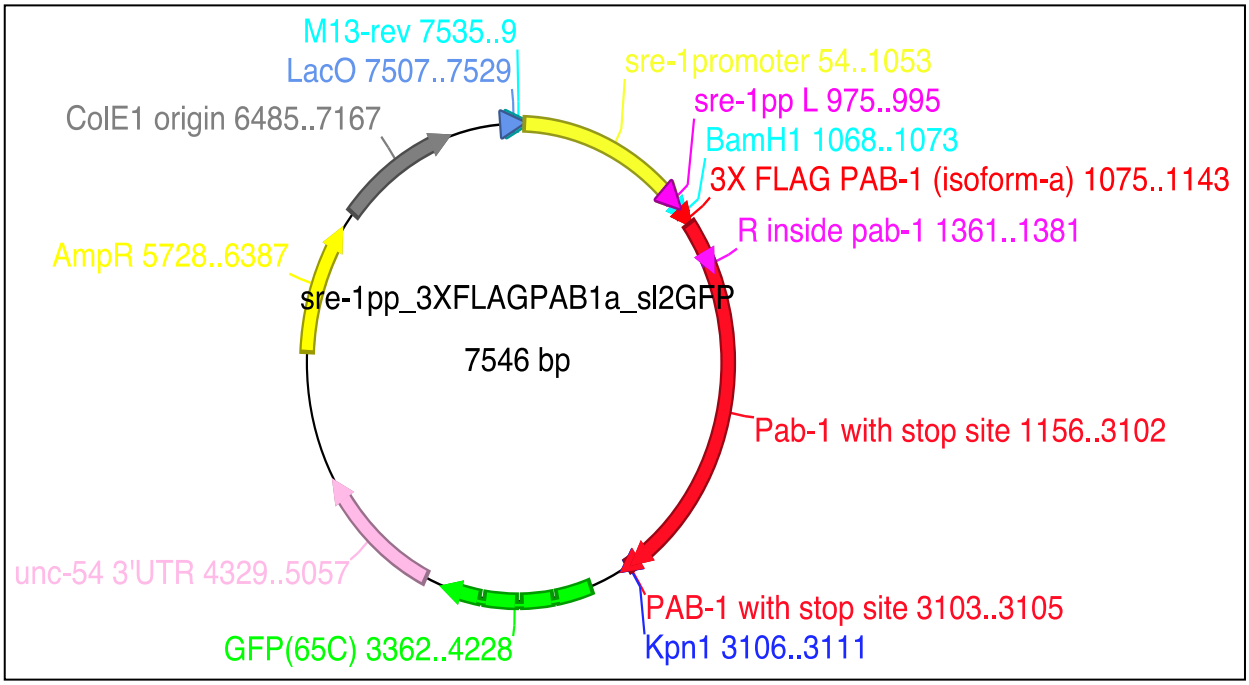

Figure 3, **Map of the vector construct to be inserted into *Caenorhabditis elegans***. Red map region represents the insert, *3xFLAG::PAB-1*. Yellow map region prior to the insert represents the *sre-1* promoter; the ampicillin resistance gene is also marked in yellow. Bam H1 and Kpn1 are each restriction enzymes: sites on the plasmid construct indicate where each of these enzymes cuts the DNA. GFP stands for green fluorescent protein.

References

ADLL, ADLR. (2014) Retrieved April 29, 2014, from http://www.wormatlas.org/neurons/Individual%20Neurons/ADLframeset.html

Barth, A. L., Dugas, J. C., & Ngai, J. (1997). Noncoordinate expression of odorant receptor genes tightly linked in the zebrafish genome. *Neuron*, *19*(2), 359-369.

Bono, M. D., Tobin, D. M., Davis, M. W., Avery, L., & Bargmann, C. I. (2002). Social feeding in *Caenorhabditis elegans* is induced by neurons that detect aversive stimuli. *Nature*, *419*(6910), 899-903.

Chan, M. S., Medley, G. F., Jamison, D., & Bundy, D. A. (1994). The evaluation of potential global morbidity attributable to intestinal nematode infections. *Parasitology*, *109*(03), 373.

Chao, M. Y., Komatsu, H., Fukuto, H., Dionne, H. M., & Hart, A. (2004). Feeding status and serotonin rapidly and reversibly modulate a *Caenorhabditis elegans* chemosensory circuit. *Proceedings of the National Academy of Sciences*, *101*(43), 15512-15517.

Crompton, D., & Nesheim, M. (2002). Nutrional impact of intestinal helminthiasis during the human life cycle. *Annual Review of Nutrition*, *22*, 35-59.

Fujiwara, M., Sengupta, P., & Mcintire, S. L. (2002). Regulation of body size and behavioral state of *C. elegans* by sensory perception and the *egl-4* cGMP-dependent protein kinase. *Neuron*, *36*(6), 1091-1102.

Hallem, E. A., & Sternberg, P. W. (2008). Acute carbon dioxide avoidance in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences*, *105*(23), 8038-8043.

Hallem, E. A., Fox, A. N., Zwiebel, L. J., & Carlson, J. R. (2004). Olfaction: mosquito receptor for human-sweat odorant. *Nature*, *427*(6971), 212-213.

Hotez, P. J., Brindley, P. J., Bethony, J. M., King, C. H., Pearce, E. J., & Jacobson, J. (2008). Helminth infections: the great neglected tropical diseases. *Journal of Clinical Investigation*, *118*(4), 1311-1321.

Hotez, Peter J. (2013). Forgotten people, forgotten diseases: the neglected tropical diseases and their impact on global health and development. Retrieved from http://www.eblib.com

Larsson, M. C., Domingos, A. I., Jones, W. D., Chiappe, M., Amrein, H., & Vosshall, L. B. (2004). Or83b encodes a broadly expressed odorant receptor essential for drosophila olfaction. *Neuron*, *43*(5), 703-714.

Marella, S., Mann, K., & Scott, K. (2012). Dopaminergic modulation of sucrose acceptance behavior in drosophila. *Neuron*, *73*(5), 941-950.

Reisenman, C. E., Lee, Y., Gregory, T., & Guerenstein, P. G. (2013). Effects of starvation on the olfactory responses of the blood-sucking bug *Rhodnius prolixus*. *Journal of Insect Physiology*, *59*(7), 717-721.

Root, C., Ko, K., Jafari, A., & Wang, J. (2011). Presynaptic facilitation by neuropeptide signaling mediates odor-driven food search. *Cell*, *145*(1), 133-144.

Sambongi, Y., Nagae, T., Liu, Y., Yoshimizu, T., Takeda, K., Wada, Y., et al. (1999). Sensing of cadmium and copper ions by externally exposed ADL, ASE, and ASH neurons elicits avoidance response in *Caenorhabditis elegans*. *Neuroreport*, *10*(4), 753-757.

Sawin, E. R., Ranganathan, R., & Horvitz, H. (2000). *C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron*, *26*(3), 619-631.

Sciacca, J., Forbes, W. M., Ashton, F. T., Lombardini, E., Gamble, H., & Schad, G. A. (2002). Response to carbon dioxide by the infective larvae of three species of parasitic

nematodes. *Parasitology International*, *51*(1), 53-62.

Sengupta, P. (2013). The belly rules the nose: feeding state-dependent modulation of peripheral chemosensory responses. *Current Opinion in Neurobiology*, *23*(1), 68-75.

Stepek, G., Buttle, D. J., Duce, I. R., & Behnke, J. M. (2006). Human gastrointestinal nematode infections: are new control methods required?. *International Journal of Experimental Pathology*, *87*(5), 325-341.

Takayama, J., Faumont, S., Kunitomo, H., Lockery, S. R., & Iino, Y. (2009). Single-cell transcriptional analysis of taste sensory neuron pair in *Caenorhabditis elegans*. *Nucleic Acids Research*, *38*(1), 131-142.

The technology behind TOPO® Cloning. (2014). Retrieved May 1, 2014, from http://www.lifetechnologies.com/us/en/home/life-science/cloning/topo/topo-resources/the-technology-behind-topo-cloning.html

van der Linden, A. M., Wiener, S., You, Y., Kim, K., Avery, L., & Sengupta, P. (2008). The *egl-4* PKG acts with *kin-29* salt-inducible kinase and protein kinase A to regulate chemoreceptor gene expression and sensory behaviors in *Caenorhabditis elegans*. *Genetics*, *180*(3), 1475-1491.

Waggoner, L., Hardaker, L. A., Golik, S., & Schafer, W. (2000). Effect of a neuropeptide gene on behavioral states in *Caenorhabditis elegans* egg-laying. *Genetics*, *154*, 1181-1192.

White, J. G., Southgate, E., Thomson, J. N., & Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *314*(1165), 1-340.

Zwietering, M., Jongenburger, I., Rombouts, F., & Riet, K. V. (1990). Modeling of the bacterial growth curve. *Applied and Environmental Microbiology*, *1*, 1875-1881.