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Roles of ubiquitin and stress in diacetyl chemosensation of C. elegans

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Food stress and ubiquitin-mediated olfactory protein trafficking dynamics in *C. elegans*

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BACKGROUND

Ubiquitin is a small protein that can be attached to other proteins in a cell, tagging them for destruction. The process of adding ubiquitin to a protein substrate (ubiquitination), and the subsequent trafficking and degradation of this substrate, is a principle regulator of the abundance and activity of many proteins across all forms of life. We are examining the role and dynamics of this regulatory system in the olfactory neurons of the model organism *Caenorhabditis elegans*, specifically the **olfactory receptor protein ODR-10**, which allows the worm to detect diacetyl, a volatile compound that is produced by the bacteria the worm eats. Without ODR-10, worms cannot properly perform "**chemotaxis**"—detection and movement towards food (Bargmann et al., 1996).

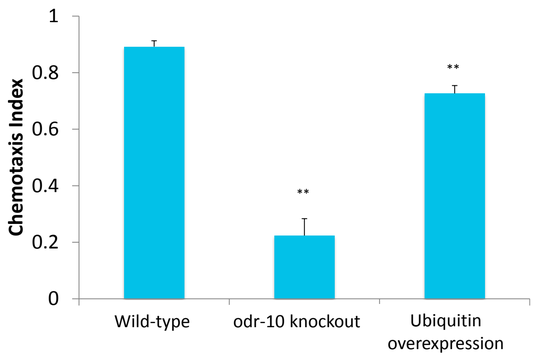


Figure 1. Chemotaxis is impaired in worms which overexpress ubiquitin.

The ubiquitin-mediated degradation pathway is known to regulate other cell-surface receptors in neurons (Kowalski et al., 2011), and by testing the diacetyl-sensing ability of worms with mutations in this pathway, we found that abnormal ubiquitination in the ODR-10 expressing neuron ("AWA") leads to reduced diacetyl detection in the worm (Fig. 1) , suggesting that the ubiquitin pathway is indeed involved in regulating ODR-10 (Fig. 2).

To further characterize how the ubiquitin degradation pathway functions to regulate the worm's sense of smell and the trafficking of ODR-10, we put worms with ubiquitin pathway mutations under conditions of **food stress** and compared both their food-seeking behavior and ODR-10 abundance/localization within the AWA neuron to the wild-type strain.

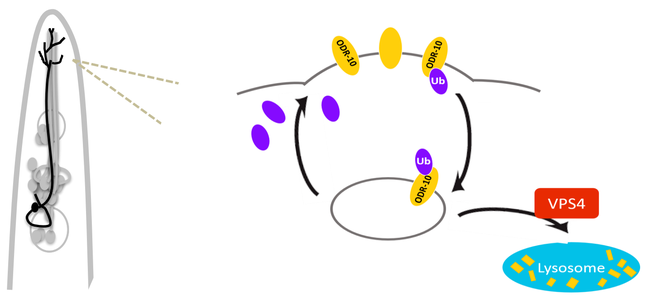
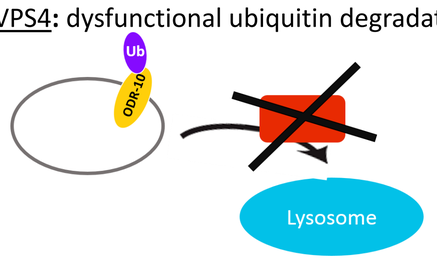


Figure 2. Representation of the putative regulation of ODR-10 by ubiquitin. VPS4 is a protein that allows ubiquitinated proteins to enter the lysosome for degradation. Such systems are constantly acting to regulate protein amounts.

Methodology

Transgenic Worms: Our genetic modifications are driven by the ODR-10 promoter P*odr-10,* which leads to transgene expression only when and where ODR-10 is normally expressed in the AWA neuron. Both of our experimental genotypes represent abnormal functionalities of the ubiquitin-mediated degradation pathway (Fig. 3)

DN-VPS4**:** dysfunctional ubiquitin degradation



Myc-Ub: overexpression of ubiquitin

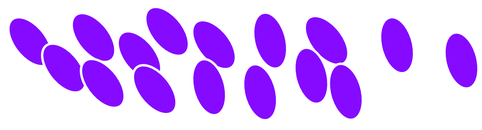
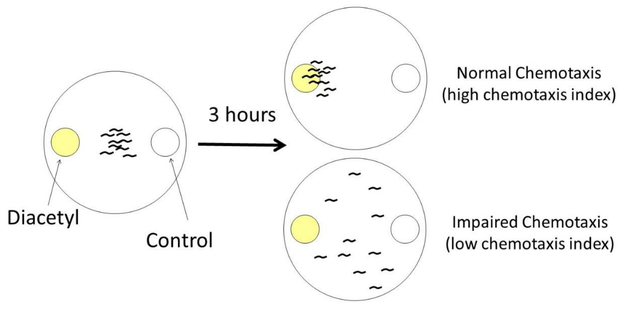


Figure 3. Representation of the effects of our two mutant transgenes on the ubiquitin-degradation system. DN-VPS4 causes the expression of a dysfunctional version of the recycling intermediate VPS4, preventing ubiquitinated proteins from entering the lysosome for degradation. Myc-Ub causes an over-expression of the ubiquitin protein, which should lead to increased ubiquitination of proteins regulated by this pathway.

Behavioral Assay: 150 worms were added to the center of a dish with a spot of diacetyl on one side and ethanol (odorless to worms) on the other. After 2.5 hours, the locations of the worms were recorded and used to find the chemotaxis index—the fraction of worms found at the diacetyl (Fig. 4). Wild-type worms have a chemotaxis index of around 0.9, meaning that nearly all of the plated worms are found at the diacetyl.

Figure 4. The chemotaxis assay, which is conducted on 100mm diameter petri dishes with a surface of agarose gel. Both the diacetyl and control spots also contain a paralytic agent which halts the worms upon reaching it



Discussion

Our results from last year showed that ubiquitin-mediated degradation is important for proper regulation of ODR-10. This year, we have shown that when the worms are starved, both wild-type and mutant animals were equally able to detect diacetyl, while imaging data have shown a buildup of ODR-10 mislocalized in the cell body rather than the cilia. We hypothesize that despite this buildup, there is enough properly functioning ODR-10 in the cilia to maintain normal chemotaxis ability. Preliminary behavioral data of the desensitization assays suggest there is a significant difference between the wild-type and mutant strains, however more testing is required. We also see buildup of ODR-10::GFP in the cell body of desensitized DN-VPS4 mutants, suggesting they respond to a different food stress by the same mechanism of increasing recycling rates of ODR-10.

RESULTS

Starved

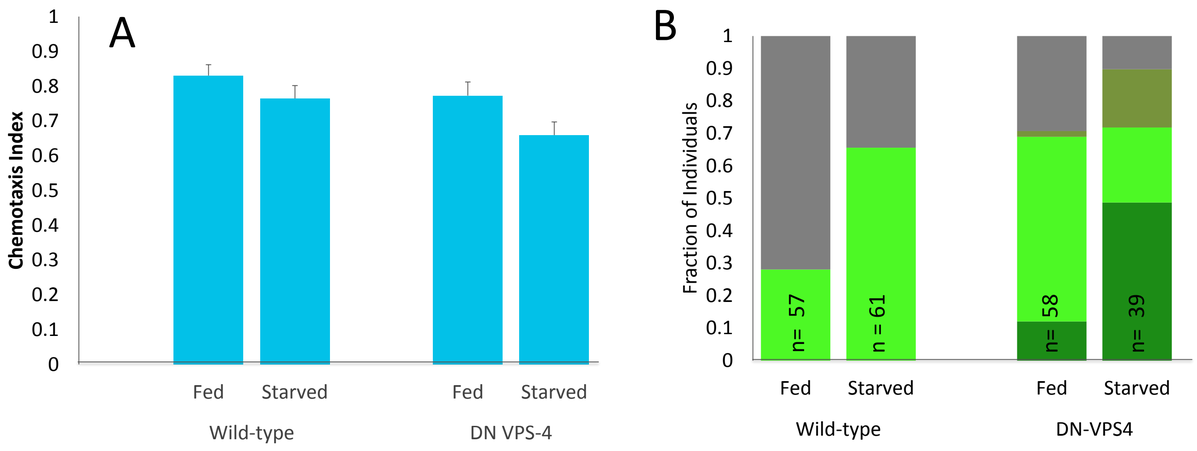


Figure 6. (A) Chemotaxis indexes of Wild-type and DN VPS-4 worms under fed/starved conditions. (B) ODR-10::GFP +/- fluorescence assay results. See figure 5 for key.

Desensitized

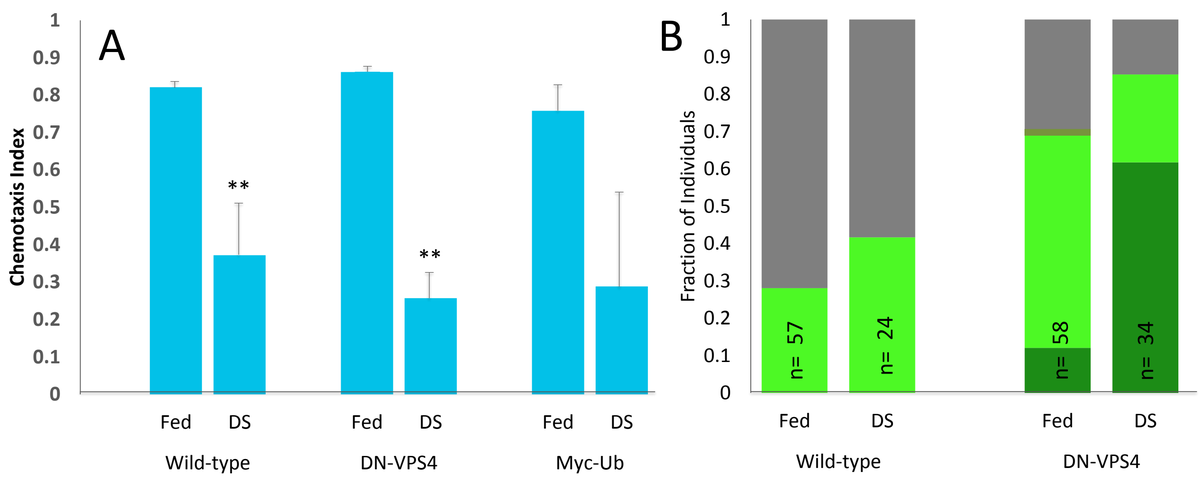


Figure 7. (A) Chemotaxis indexes of Wild-type,DN VPS-4 and Myc-Ub worms under desensitized conditions. (B) ODR-10::GFP +/- fluorescence assay results. See figure 5 for key.

In the starvation assays, worms were deprived of food for 12-18 hours prior to assaying. We hypothesized that receptor recycling rates will increase under starved conditions and be impaired under the DN-VPS4 mutation, causing chemotaxis ability to reduce heavily. While no significant differences in behavior were observed, the fraction of worms with visible ODR-10::GFP in their cilia is higher than wild-type in both fed and starved DN-VPS4. N=18 for the behavioral assays for both strains.

β-arrestin is a protein responsible for shutting off a surface receptor by dragging it into the membrane. It has been shown in other systems that β-arrestin acts to provide a target for the degradation machinery (Herrador et. al., 2010), and we thought this would also be used in *C. elegans* when regulating ODR-10. We desensitized the worms by exposing them to pure diacetyl for 1 hour prior to assaying to test their ability to desensitize. Preliminary data from behavioral assays show an expected difference between the unexposed animals and the exposed animals. Data for the N=11 for the wild-type behavioral assays and N=7 for both mutant strains.

All statistics were done using the Student's t-test, $p<0.005$ with error bars set to SEM.

ODR:10::GFP +/- Fluorescence Assay: We used worms expressing ODR-10 tagged with Green Fluorescent Protein (GFP) to visualize the localization and relative amount of ODR-10 within the AWA neuron via fluorescent microscopy. Two strains were visualized under two food stress states and compared: CX3344 (effectively wild-type) and CLD17, which expresses a dysfunctional version of the ubiquitin-pathway protein VPS-4 (Fig. 2). Worms were visualized on a Leica DMi6000 at specific settings (500ms exposure, gain = 10) and visibility of fluorescence in the sensory cilia and cell body were marked as positive or negative (Fig. 5).

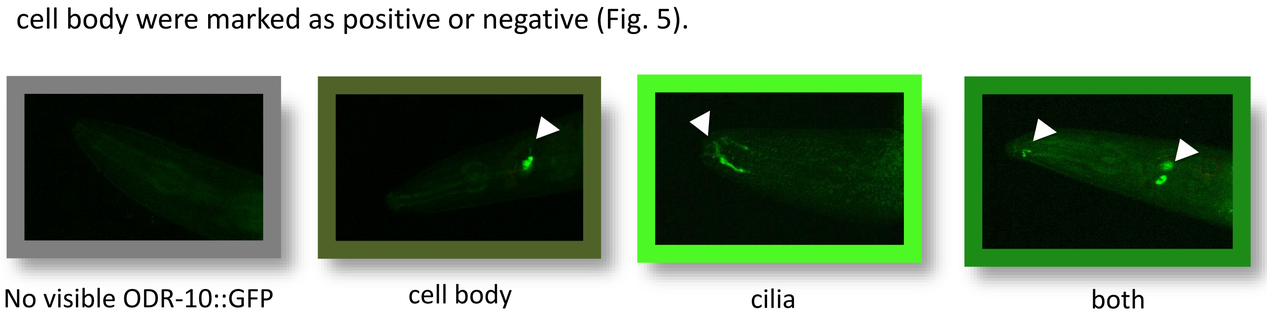


Figure 5. Representative images of each of the 4 possible states of fluorescence in the ODR-10::GFP +/- assay. Triangles indicate locations of fluorescence. The colors of the image borders correspond to the colors in figures 6B and 7B and serve as a key for these graphs.

Future Directions

Based on these data, there are many ways we can continue to explore this pathway. We can continue to study chemotaxis ability and imaging simultaneously on strains that have other mutations along the ubiquitin-mediated degradation pathway, such other enzymes that regulate ubiquitin or the ESCRT proteins, (E2/E3, pre-β-arrestin kinases) or we could stress the worms in different ways (heat, anoxic, osmolarity) and observe their ODR-10 abundance/localization and chemotaxis abilities.

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