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Importance of ubiquitin-mediated degradation on diacetyl chemosensation in c. elegans

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Effects of ubiquitin-mediated degradation on diacetyl chemosensation in *Caenorhabditis elegans*

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Abstract

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. We are examining the role of this system in the olfactory neurons of the model organism *C. elegans*. We hypothesize that the ubiquitin-mediated degradation system is involved in the regulation of the abundance of the olfactory receptor ODR-10, which detects the small molecule diacetyl. In this study, we are comparing the behavioral responses of transgenic strains whose ubiquitin system was genetically altered with those of wild-type animals. We are also using fluorescent imaging to detect changes in ODR-10 abundance. Our preliminary results suggest that normal ubiquitination is involved in the maintenance of diacetyl detection in the worm.

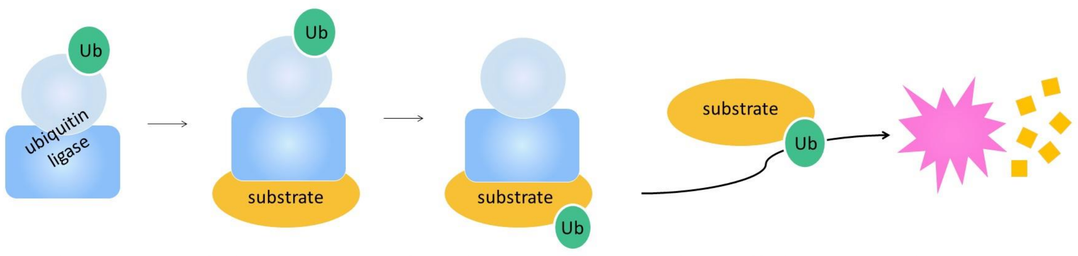
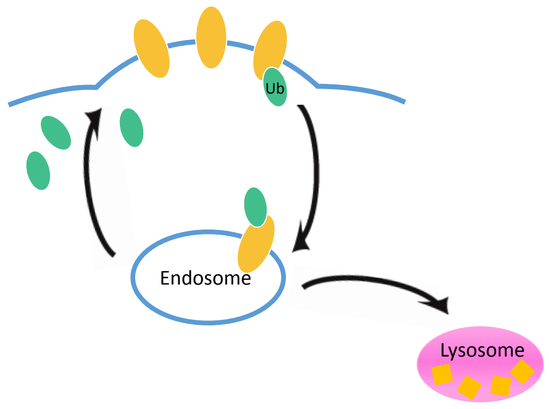


Figure 1. The process of ubiquitination of a substrate, tagging it for trafficking towards eventual destruction.

Introduction

Ubiquitin-mediated degradation is a system that negatively regulates the abundance of a protein by "tagging" it with ubiquitin, marking it for destruction (Fig. 1). This and other systems of downregulation act in balance with protein synthesis, allowing the cell to maintain protein levels or modulate them quickly and precisely through the attenuation of formation and degradation rates.



The synaptic receptor GLR-1 is regulated by ubiquitin-mediated degradation in *C. elegans* (Kowalski et al, 2011).

We hypothesize that a similar mechanism may be used to regulate sensory receptors such as ODR-10 (Fig. 2).

Figure 2. GLR-1 is ubiquitinated and subsequently endocytosed, where it can either be de-ubiquitinated and reuptake into the cell surface, or continue to the lysosome for destruction. We hypothesize that the ODR-10 receptor is regulated by ubiquitin in a similar manner.

Transgenic animals

The *odr-10* gene is only expressed in the **AWA neuron** of the worm (Fig. 3).

Creating artificial DNA sequences that begin with the *odr-10* promoter allows us to drive the expression of any protein specifically in the AWA neurons. Experimental Strains:

- **CX3344 (kyIs53) P*odr-10*::*odr-10*::GFP**
- **CLD 8 (dahEx8) P*odr-10*::GFP**
- **CLD 11 (dahEx11) P*odr-10*::MUb**

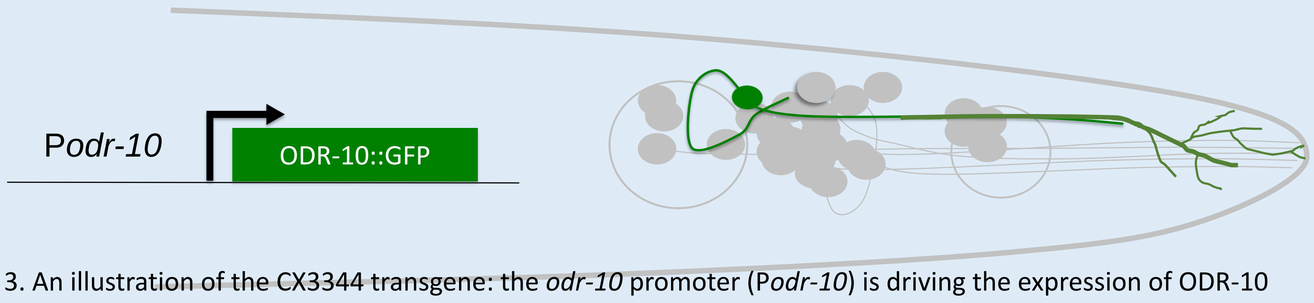
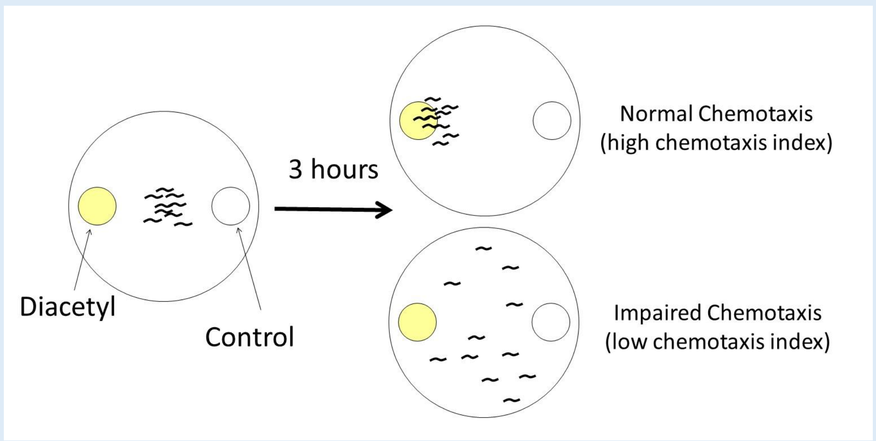


Figure 3. An illustration of the CX3344 transgene: the *odr-10* promoter (P*odr-10*) is driving the expression of ODR-10 (::ODR-10) fused to the GFP protein (::GFP). The ciliated AWA neuron, which is the only cell in which the ODR-10 receptor is expressed, and therefore the only cell in which our transgenes are expressed. When a worm this transgene, the AWA neuron glows green under fluorescence.

Chemotaxis behavioral assay

C. elegans senses and moves towards sources of diacetyl, a volatile compound generated by the bacteria it consumes. This behavior is dependent on **the diacetyl receptor, ODR-10**.



Chemotaxis Index = $\frac{\text{\# worms at diacetyl - \# worms at negative control}}{\text{total worms}}$

Figure 4. Above, the seven-transmembrane domain olfactory receptor ODR-10. Left, the experiment we use to assay the chemotaxis (movement in response to smells) of our transgenic worms. Bottom left, the equation we use to quantify the behavioral response of a transgenic strain to diacetyl.

Fluorescence imaging

GFP stands for **Green Fluorescent Protein**, so named for the green light it emits under fluorescence. The introduction of GFP driven by cell-specific promoters is a common method of expression localization and quantification in the transparent *C. elegans*. This method provided evidence for the control of GLR-1 abundance by ubiquitin-mediated degradation (Fig. 5) , and could provide us with similar evidence for ODR-10.

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Figure 5. From Burbea et al, 2006. (A) The expression of GLR-1 in the ventral nerve cord of *C. elegans* as visualized by fluorescence microscopy. Brightness of spots corresponds to the abundance of GFP driven by the *glr-1* promoter. (B) The fluorescence intensity of P*glr*-1::GFP is greatly diminished by the expression of myc-tagged ubiquitin (MUb), supporting the theory that ubiquitin negatively regulates the abundance of GLR-1 in *C. elegans*.

Results

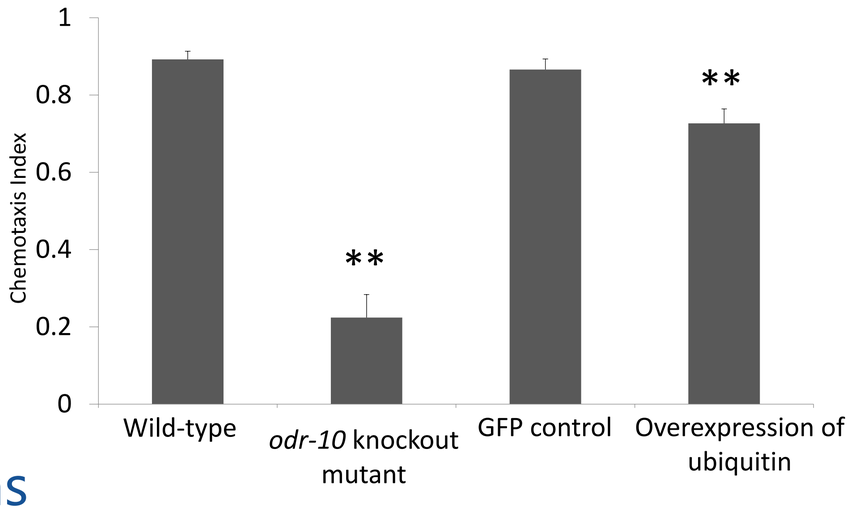

Figure 6. Chemotaxis indices of experimental strains (WT, ky225, dahEx8, dahEx11), with SEM error bars, n=18, $p < 0.01$. The *odr-10* knockout mutant shows highly impaired chemotaxis, whereas the strain overexpressing ubiquitin shows moderately impaired chemotaxis.

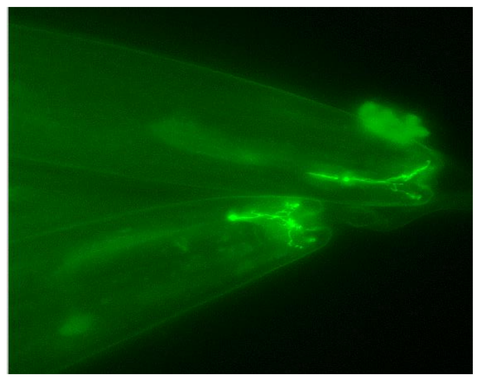

Figure 7. A fluorescence microscope image of GFP expression in a CX3344 (P*odr-10*::*odr-10*::GFP) worm. The GFP is being expressed in the AWA cilia, as driven by the P*odr-10* promoter. This image was taken at 63x magnitude.

Future Directions

- Our current behavioral data suggests that the ubiquitination process is necessary for proper regulation of the ODR-10 receptor.
- We will seek more or less pronounced responses of our transgenic strains at lower diacetyl concentrations through generation of a dose response curve.
- Use of fluorescence imaging and Western Blotting to assess ODR-10 abundance under AWA-specific ubiquitin overexpression

References

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