Characterization of Neural Phenotypes of *ubc-1(R7Q)* and *ubc-1 (R11Q)* mutants in *C. elegans*

Harjas Dhillon, *Occidental College* | Dr. Renee Baran, *Occidental College*

I. Introduction

The ubiquitination and degradation of proteins is an essential mechanism in the regulation of protein levels, which is crucial in cellular processes such as cell-cycle progression, signal transduction, and protein quality control. Ubiquitination also plays a role in neural development and function. The process of targeting a substrate for proteasomal degradation is mediated by a cascade that includes ubiquitin-activating E1 enzymes, ubiquitin-conjugating E2 enzymes and E3 ubiquitin ligases (Segref & Hoppe, 2008). Multiple subtypes of each subunit are coded in invertebrate and vertebrate genomes. *Ubc-1* is an E2 ubiquitin-conjugating enzyme found in *C. elegans* (WormBase). The absence of *ubc-1*(complete loss of function) affects multiple tissues and causes poor health, low brood size, lack of coordination, and synaptic defects (P. Jin, M. Zhen, personal communication). Complete loss of its human homolog *Ube2a* is associated with multiple defects including failure of DNA repair and is associated with X-linked intellectual disorder (Budny et al. 2010). This project will examine the neural phenotypes of *ubc-1* (R7Q) and *ubc-1* (R11Q) mutants in *C. elegans* using GFP transgenes to help determine if they cause similar defects in *C. elegans* and could serve as a model for human Ube2a X-linked disability.

References

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Segref, A., & Hoppe, T. (2009). Think locally: Control of ubiquitin-dependent protein degradation in neurons. *EMBO Reports*, *10*(1), 44–50.

Valimberti, I., Tiberti, M., Lambrughi, M., Sarcevic, B., & Papaleo, E. (2015). E2 superfamily of ubiquitin-conjugating enzymes: Constitutively active or activated through phosphorylation in the catalytic cleft. *Scientific Reports, 5*

Wormbase.org

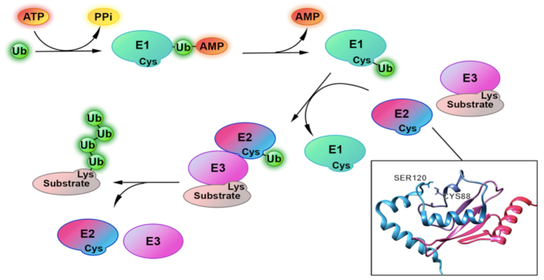


Fig 1. E2 ubiquitin-conjugating enzymes work with E3 ubiquitin ligase to attach a ubiquitin chain to a substrate, targeting the substrate for degradation (Valimberti et al. 2015).

II. Characterizing Synaptic Phenotype of *ubc-1 (R7Q) and (R11Q) mutants*

Transgenic markers juIS1 (Punc-25::GFP, a marker for GABAergic synapses, and hpls201 (Pceh-10::GFP), a marker for the RID interneurons will be crossed into the mutant strains to study their neural phenotypes with fluorescence microscopy. In *ubc-1* null mutations (complete loss-of-function) expressing these transgenic markers, the puncta are not as bright and gaps between them are more variable relative to the wildtype phenotype (Jin and Zhen, unpublished data).

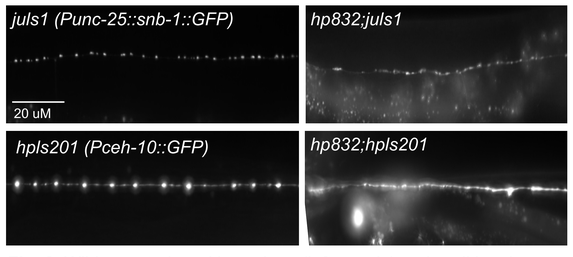


Fig. 2. Wildtype strains with markers (left panels) and traditional *ubc-1* mutants with markers (right panels) have distinct and identifiable differences in phenotype. Each puncta represents the synaptic vesicles at a single synapse (Jin & Zhen, unpublished data). Scale bar 20 uM.

III. Constructing Strains with Transgenic Markers

The following series of crosses will be carried out to create a strain of *ubc-1* mutants expressing the juIs1 marker to visualize synapses. A red fluorescent transgene marker located on the same chromosome as *ubc-1* will be used to balance the mutant allele. The crosses detailed below specify RBNO2 mutants and the juIS1 marker, but will be repeated with the hpIs201 marker. RBNO1 mutants will also be crossed with both transgenic markers.

P0: juIS1 (III) / juIS1 (III) ♂ X red fluorescent marker (IV) / red fluorescent marker (IV)

juIS1 (snb-1::GFP) males will be crossed with a red fluorescent marker located on the same chromosome as *ubc-1*.

F1: red fluorescent marker (IV) / + ; juIS1 (III) / +

The F1 progeny will be heterozygous for both transgenes.

P0: red fluorescent marker (IV) / + ; juISI(III) / + ♂ X RBNO2 (IV) / RBNO2 (IV)

Males from the initial cross will be mated with RBNO2 hermaphrodites.

F1: red fluorescent marker (IV) / RBNO2 (IV) ; juIS1 (III) / wt

I will select progeny with both the red fluorescence marker and juIS1 (GFP green) phenotypes using a fluorescence microscope. The selected animals will be placed on individual plates and allowed to generate self-progeny.

F2: RBNO2 (IV) / RBNO2 (IV) ; juIS1 (III) / juIS1 (III)

F2 progeny without the balancer marker will be selected using a dissecting microscope. 1/16 will be homozygous for both the *ubc-1* mutant allele and the juIs1 transgenic marker.

IV. Genotyping *ubc-1 (R7Q) and (R11Q) mutants*

In addition, I will be using amplification-refractory mutation system (ARMS) PCR to genotype the constructed strains. ARMS is able to detect single nucleotide polymorphisms (SNPs) without sequencing. I analyzed WT and mutant sequences and designed PCR primers specific to each *ubc-1* allele using ApE DNA analysis tools.

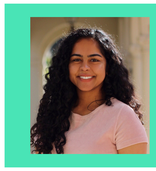
ARMS PCR primer criteria:

- WT forward primer 3' end ends on SNP site
- Mutant forward primer 3' end ends on SNP site
- Additional mutations in the sequence to enhance primer specificity
- Common reverse primer located on the opposite strand

WT fwd primer: cccagccgtagAcgtttgatgAGA

RBNO2 fwd primer: cagccgtagGcgtttgatgCAG

Common rev primer: gggcatttttttgtgcttttcgaccg



Harjas Dhillon
Occidental College
Biology Department
hdhillon@oxy.edu

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