May 19th, 12:00 PM - 3:00 PM

Regulation of AMPA-type glutamate receptor homolog GLR-1 by ERAD ubiquitin ligases in C. elegans

Sam Witus
Western Washington University

Regulation of the glutamate receptor, GLR-1, by ERAD ubiquitin ligases in *C. elegans*

Sam Witus and Lina Dahlberg

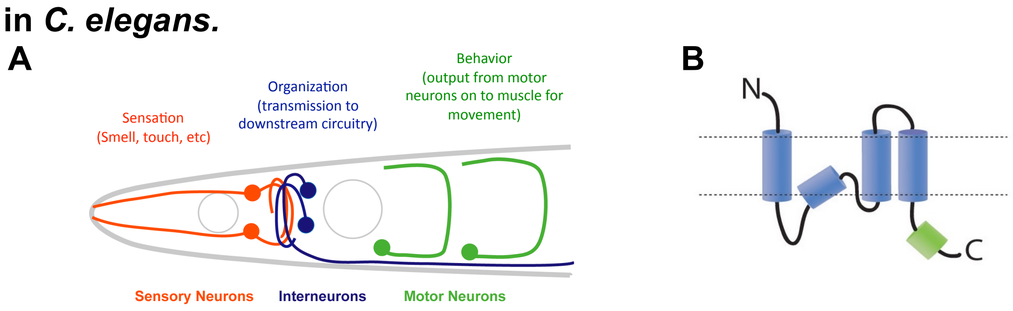
Department of Biology, Western Washington University
516 High St, Bellingham, WA 98225

Abstract

Endoplasmic reticulum-associated degradation (ERAD) maintains cellular health by removing misfolded proteins from the endoplasmic reticulum (ER). ERAD is ubiquitin-dependent, and ubiquitination of target proteins can be catalyzed by ER-resident E3 ubiquitin ligases. In *C. elegans*, genes for three putative ERAD E3 ubiquitin ligases have been identified: *hrd-1*, *hrdl-1*, and *marc-6 (HRD-1, GP78/AMFR, and MARCH-6* in mammalian systems) (1). In *C. elegans,* these three genes cooperate to maintain the overall health of animals during ER stress. We are testing the roles of *hrd-1*, *hrdl-1*, and *marc-6* in the neurons of *C. elegans*.

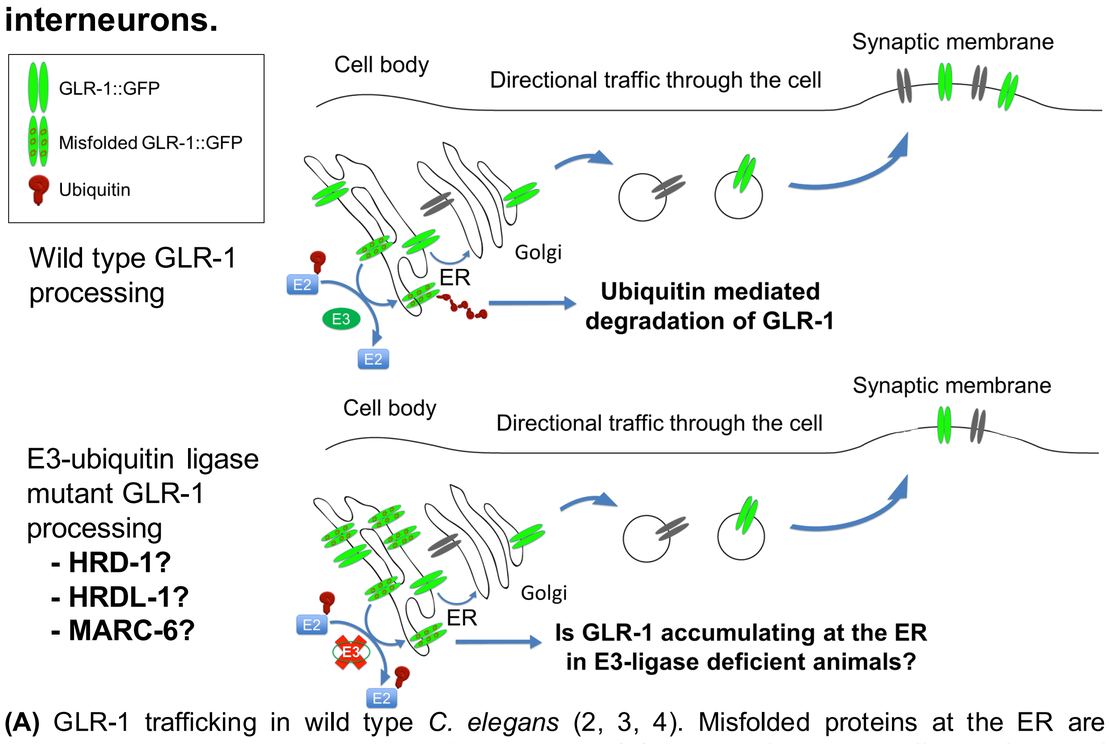
GLR-1 is a glutamate receptor that is expressed in a subset of interneurons in *C. elegans*. It is homologous to human AMPA-type glutamate receptors, which are central for the processes of learning and memory. GLR-1 that is tagged with GFP (GLR-1::GFP) recapitulates normal biochemical GLR-1 function and allows observation of its abundance and localization in live animals (2, 3, 4, 5). Animals harboring mutations in the ERAD E3 ligases *hrd-1* and hrdl-*1* have increased GLR-1::GFP abundance compared to wild-type animals. *hrd-1* and *hrdl-1* mutants also show defects in GLR-1::GFP localization. Double mutant analysis suggests that *hrdl-1* and *hrd-1* do not act redundantly, but could act in the same pathway. We are currently testing how a mutation in *marc-6* affects GLR-1::GFP. Future experiments will focus on determining the mechanism(s) by which GLR-1 is selected as a substrate by the ERAD E3 ligases and their associated E2 ligases.

Figure 1. GFP-tagged GLR-1 is expressed in a subset of interneurons in *C. elegans.*



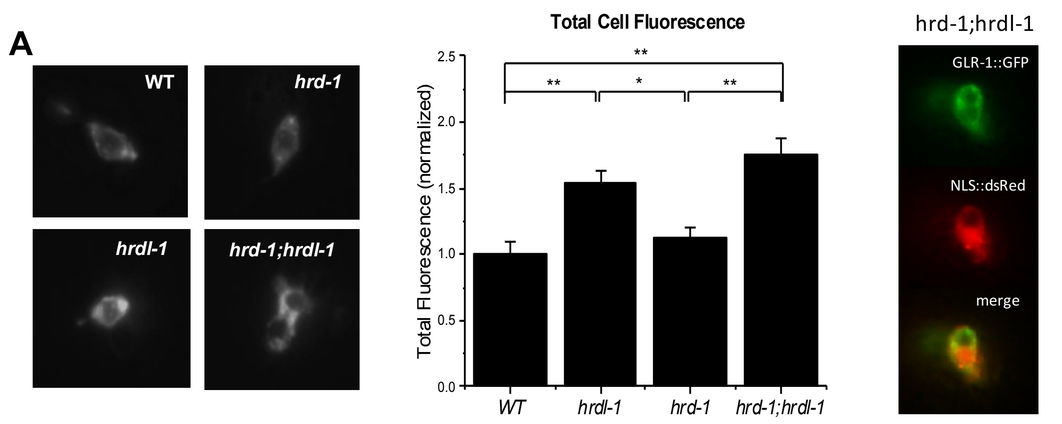
(A) Schematic diagram of neurons in the head and anterior body of the nematode, *C. elegans*. **(B)** GFP fused to the cytoplasmic C-terminal domain of GLR-1 (GLR-1::GFP) is expressed in a subset of interneurons (blue cells, in (A)) under the *glr-1* promoter (2, 3).

Figure 2. Model of GLR-1 processing and localization in interneurons.



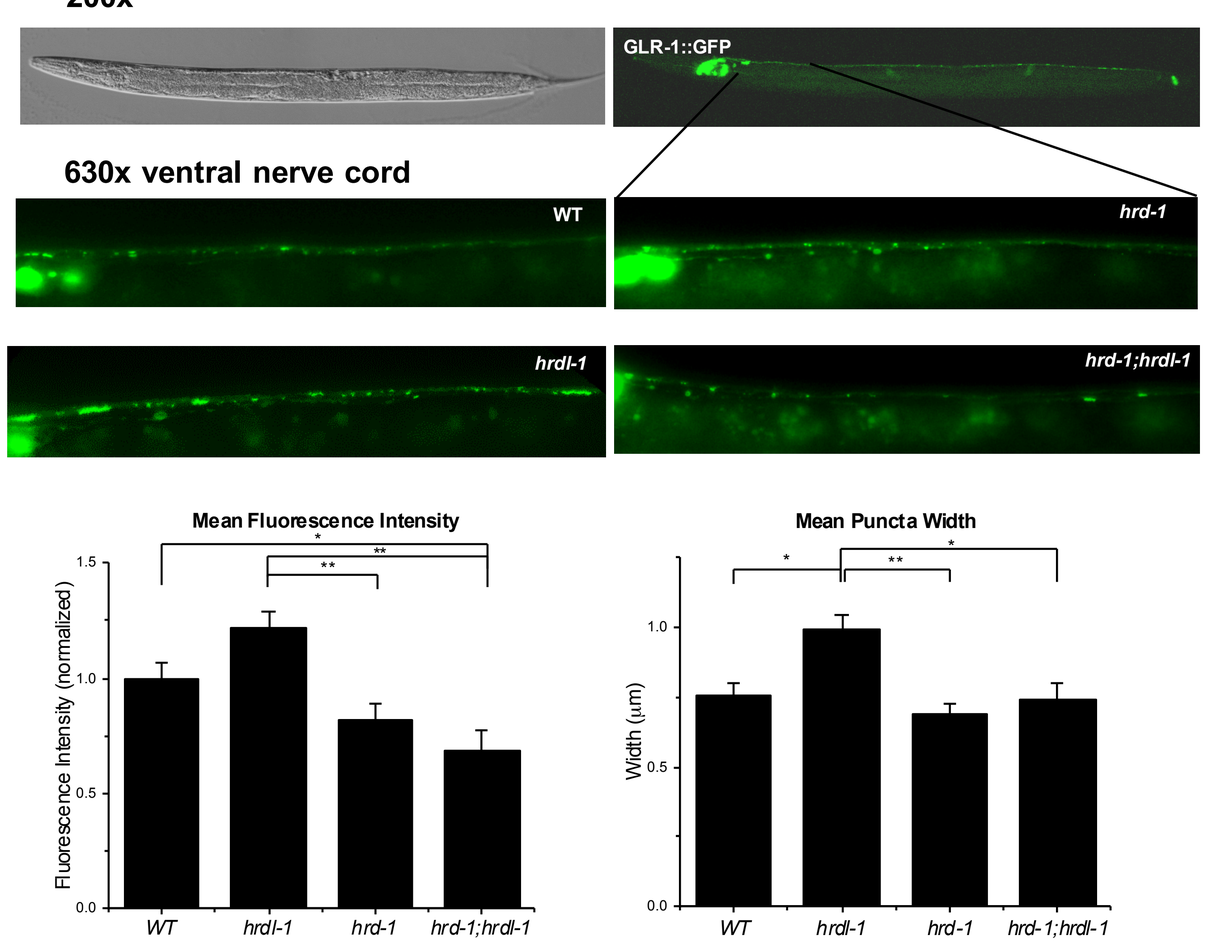
(A) GLR-1 trafficking in wild type *C. elegans* (2, 3, 4). Misfolded proteins at the ER are ubiquitinated and degraded by ERAD machinery. **(B)** Model of potential effects of loss of ERAD E3 ligases on GLR-1. In the absence of ubiquitination, misfolded substrates may be retained at the ER.

Figure 4. *hrdl-1,* but not *hrd-1*, mutants show increased GLR-1::GFP fluorescence in the PVC interneuron cell body.



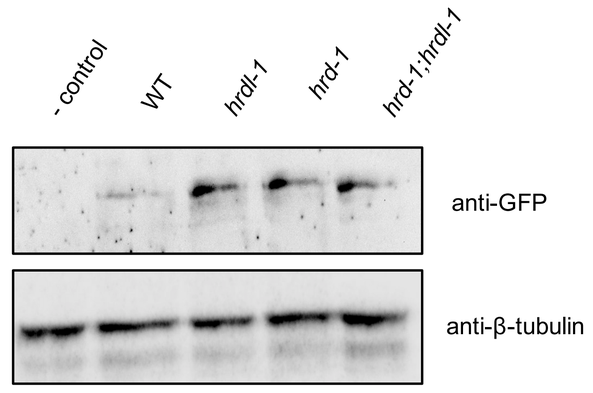
(A) Representative images of GLR-1::GFP intensity and cell morphology in PVC interneuron cell bodies of L4 wild type and E3-ligase deficient animals. **(B)** Quantification of GLR-1::GFP mean fluorescence intensity for genotypes showed in (A) (WT n=29, *hrd-1* n=30, *hrdl-1* n=31, *hrd-1;hrdl-1* n=23.) Error bars represent SEM and p-values are calculated by Tukey-Kramer post test.

Figure 3. Deletion of *hrdl-1* and *hrd-1* have different effects on the accumulation of GLR-1::GFP in the ventral nerve cord.



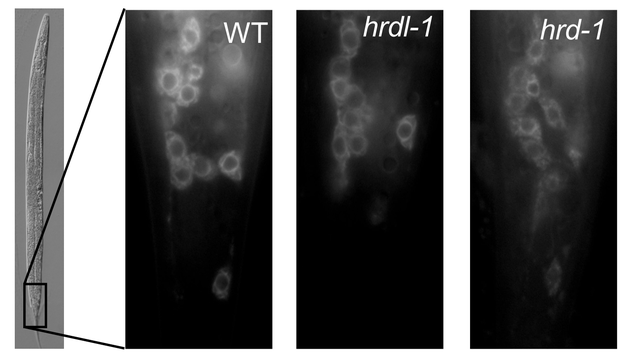
(A) L4 stage *C. elegans* imaged using DIC (left) and GFP fluorescence (right) microscopy. GLR-1 localizes in cell bodies and the ventral nerve cord (VNC) along the side of the animal. **(B)** 630x magnification of the anterior VNC used for GLR-1::GFP quantification. **(C)** *hrdl-1(gk28)* mutants display wider fluorescent puncta than wild type, *hrd-1(tm1743)*, and *hrd-1; hrdl-1* animals. **(D)** *hrd-1* and *hrd-1; hrdl-1* mutant animals have diminished punctal fluorescence compared with *hrdl-1* mutants. Wild type n=20, *hrd-1* n=24 *hrdl-1* n=31, *hrd-1;hrdl-1* n=16. Error bars represent SEM and p-values are calculated by Tukey-Kramer post test.

Figure 5. Animals with deletion mutations in *hrd-1* and *hrdl-1* E3-ligases have increased amounts of total GLR-1:GFP



Whole cell lysate was obtained from mixed staged animals with the indicated genotypes (WT, *hrdl-1, hrd-1*, or *hrdl-1; hrd-1*. Blots were incubated with anti-β-tubulin polyclonal antibodies as a loading control and anti-GFP monoclonal antibodies to probe GLR-1::GFP abundance. Lysates from animals lacking *hrd-1, hrdl-1,* or both appear to have increased amounts of GLR-1::GFP compared to the WT control.

Figure 6. Endoplasmic reticulum in ERAD E3 single mutant animals is present and localized to cell bodies, in neurons.



The ER marker TRAM-1-GFP expressed under a pan-neuronal promoter and imaged in tail neurons of L4 stage *C. elegans*. ER is visible surrounding the nucleus and throughout the soma in WT and single mutant animals. Fluorescence was not detected in the VNC of any animals.

Conclusions and future directions

Two ERAD E3 ubiquitin ligases, *hrd-1* and *hrdl-1* affect the abundance and localization of the GLR-1 in neurons. While the ER is not grossly affected in these mutants, we are investigating the mechanisms and effects of these mutations in the cell and for the animal. We are working to obtain quantitative western blotting data on GLR-1 abundance in mutant animals and to characterize animals that are mutant for the *marc-6* gene. We also plan to determine the relative amounts of GLR-1 that is fully processed in the ER and Golgi in the presence or absence of the three E3 ligases introduced here. A recent study also suggests that proteins that are not processed properly by ERAD machinery can form inclusions that do not colocalize with the ER. We hope to address the nature of the punctate accumulations of GLR-1 in *hrdl-1* mutants, and of the cell body fluorescence in *hrd-1; hrdl-1* double mutant animals.

References

(1) Sasagawa, et al. (2007) Genes to Cells, 12:1063
(2) Monteiro, et al. (2012) Mol. Biol. Cell, 23 (18)18:3647-62
(3) Rongo, et al. (1998) Cell, 94:751
(4) Burbea, et al. (2002) Neuron, 35:107
(5) Dahlberg and Juo (2014) JBC, 289 (6):3444
(6) Zhang, et al.(2015) MBoC, 26:4438

Acknowledgements

Thank you to the Ailion laboratory for the TRAM-1 reporter line (EG5748).This research was initiated in the lab of Peter Juo at Tufts University. S.W. was partly supported by the office of Research and Sponsored Programs at WWU.