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Regulation of the AMPA glutamate receptor homolog GLR-1 at the endoplasmic reticulum in C. elegans.

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Regulation of the AMPA glutamate receptor homolog GLR-1 abundance at the endoplasmic reticulum in C. elegans.

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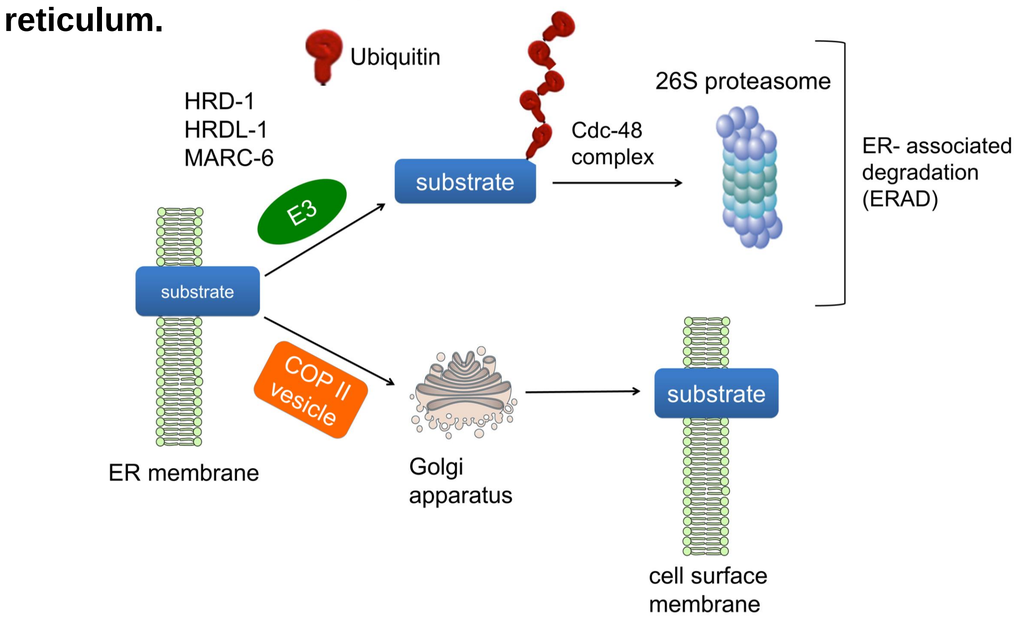
Abstract

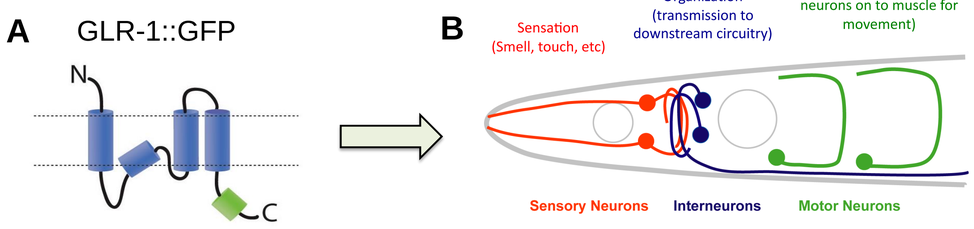
In *C. elegans,* the glutamate receptor GLR-1 functions in the nervous system to decode environmental stimuli and sensory experiences, and to regulate locomotion and the formation of long-term memory. *C. elegans* GLR-1 is homologous to mammalian glutamate receptors, and we can use this simple organism as a system to better understand the life cycle of human receptors (1). Because GLR-1 is a membrane protein, it is first assembled in the interior of a neuron, and then it is transported to the membrane at the surface of the cell so that it can receive chemical signals (glutamate) from the environment. Currently, many of the detailed cellular mechanisms that regulate the abundance of GLR-1 after it has been exported to the cell surface are known (2, 3). However, it is less clear how the abundance of GLR-1 is regulated in the interior of the cell prior to being transported to the cell surface. Before GLR-1 is exported to the cell surface it must be properly folded into its active conformation at the endoplasmic reticulum (ER). Here we investigate the role of three E3-ubiquitin ligases that function in endoplasmic reticulum associated degradation (ERAD) HRD-1, HRDL-1, and MARC-6. Using fluorescence microscopy HRDL-1 depleted C. *elegans* display wide patches of GLR-1::GFP suggesting accumulation of the protein at the ER. We also employ Western blotting to determine the relative concentrations of GLR-1 in the absence of E3-ubiquitin ligases. Our research suggests that the depletion of HRDL-1 causes accumulation of GLR-1.

Figure 1. Fates of integral membrane proteins at the endoplasmic reticulum.



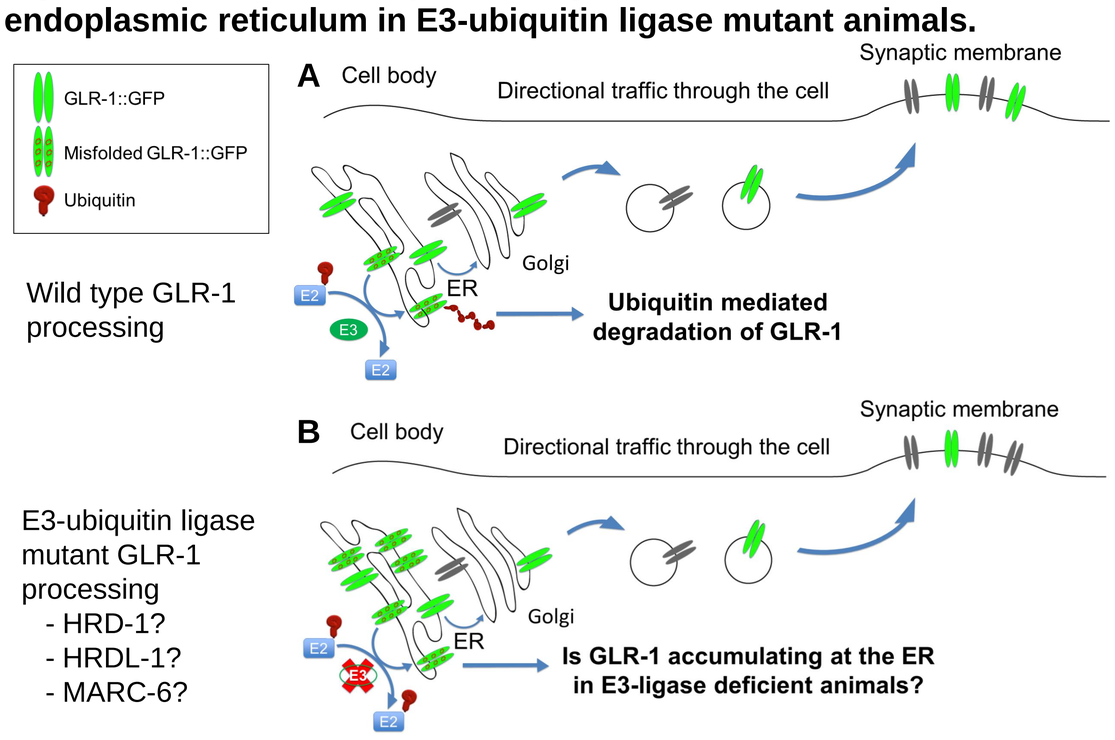
In the ER, faulty proteins are selected for and degraded in ERAD. Three ER resident E3-ubiquitin ligases that add ubiquitin to substrates and signal for their degradation through ERAD are HRDL-1, HRD-1, and MARC-6 (4).

Figure 2. GFP tagged GLR-1 is expressed in the interneurons of *C. elegans.*



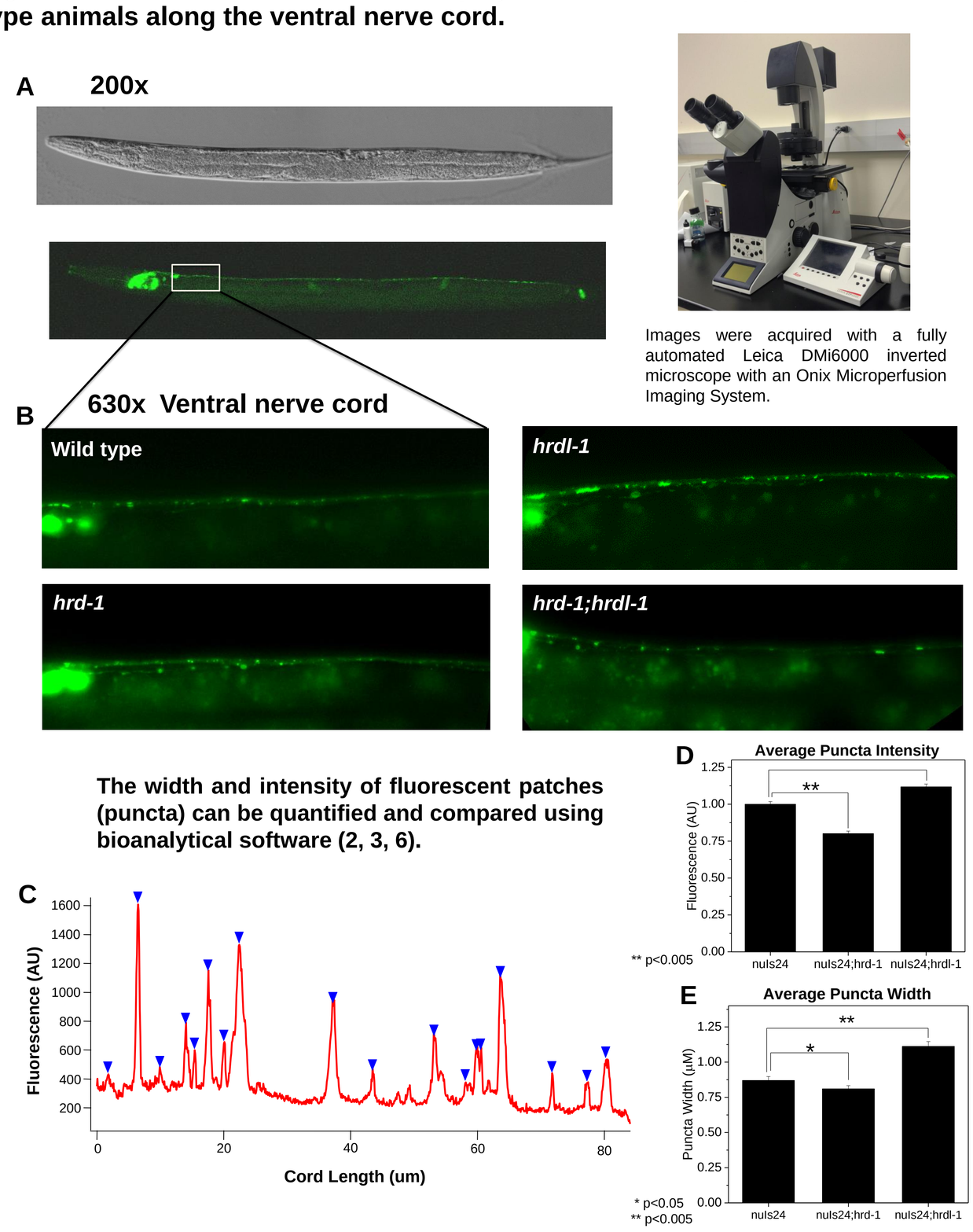
(A) GFP is fused to the cytoplasmic C-terminal domain of GLR-1. **(B)** GLR-1 is expressed in the interneurons of *C. elegans* under the *glr-1* promoter and localizes to the ventral nerve cord in interneurons (blue cells) (5, 6).

Figure 3. Model of GLR-1 processing and localization at the endoplasmic reticulum in E3-ubiquitin ligase mutant animals.



(A) GLR-1 trafficking in wild type *C. elegans*. Misfolded proteins at the ER are ubiquitinated and degraded in ERAD. **(B)** Schematic of GLR-1 accumulation at the ER in E3-ubiquitin ligase mutant animals. In the absence of ubiquitination, misfolded substrates could be retained at the ER.

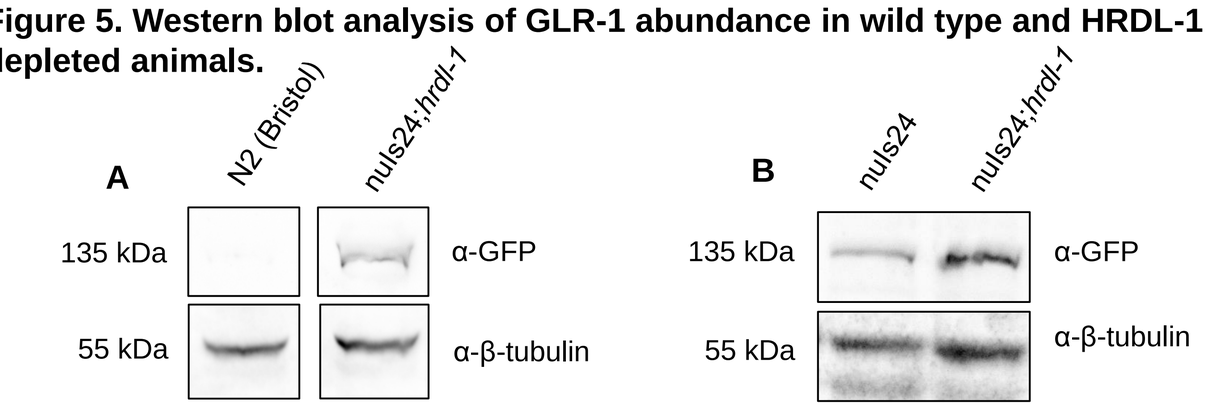
Figure 4. HRDL-1 depleted animals display wider GLR1::GFP patches than wild type animals along the ventral nerve cord.



Images were acquired with a fully automated Leica DMi6000 inverted microscope with an Onix Microperfusion Imaging System.

(A) L4 stage *C. elegans* imaged in DIC (top) and GFP fluorescence (bottom). GLR-1 is produced in the anterior neurons and localized to the ventral nerve cord (VNC) along the side of the animal. **(B)** 630x magnification of the anterior VNC used for GLR-1::GFP quantitation. **(C)** Sample of the raw data readout used for fluorescence quantitation. **(D)** HRD-1 depleted animals display dimmer fluorescence than wild type animals. HRDL-1 mutants do not. **(E)** HRDL-1 mutants display wider puncta than wild type animals. HRD-1 depleted animals display narrower puncta than wild type.

Figure 5. Western blot analysis of GLR-1 abundance in wild type and HRDL-1 depleted animals.



(A) Whole cell lysate was obtained from mixed staged animals and fixed on a nitrocellulose membrane. Blots were incubated with anti-β-tubulin polyclonal antibodies as a loading control and anti-GFP monoclonal antibodies to probe GLR-1 abundance. **(B)** HRDL-1 depleted *C. elegans* qualitatively appear to have increased amounts of GLR-1 than the positive control.

Future directions

We plan on continuing fluorescence imaging and Western blotting experiments with *hrd-1, hrdl-1, and hrdl-1;hrd-1* mutants. We are also in the process of obtaining and including *marc-6* mutants in these and subsequent experiments.

In the future, introduction of a fluorescent ER marker into these strains will enable us to visualize GLR-1 ER localization. In conjunction with fluorescence, the Endo H assay will elucidate the glycosylation profile of GLR-1 in mutant animals allowing us to quantify the relative amounts of GLR-1 that has been processed by the Golgi and how much is retained in the ER.

References

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Acknowledgements

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