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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, HRD-1, and MARC-6. Using fluorescence microscopy HRD1-1 depleted C. elegans display wide patches of GLR1::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 HRD1-1 causes accumulation of GLR-1.

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

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

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

Figure 4. HRD1-1 depleted animals display wider GLR1::GFP patches than wild type animals along the ventral nerve cord.

Figure 5. Western blot analysis of GLR-1 abundance in wild type and HRD1-1 depleted animals.

Future directions
We plan on continuing fluorescence imaging and Western blotting experiments with hrd-1, hrd-1, and hrd1-1hrd-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 conjuction 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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