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

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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, hrd-1, and marc-6 (HRD-1, GPT7AMFR, 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, hrd-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 hrd-1 have increased GLR-1::GFP abundance compared to wild-type animals. hrd-1 and hrd-1 mutants also show defects in GLR-1::GFP localization. Double mutant analysis suggests that hrd-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.

Conclusions and future directions

Two ERAD E3 ubiquitin ligases, hrd-1 and hrd-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 and 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 hrd-1 mutants, and of the cell body fluorescence in hrd-1;hrd-1 double mutant animals.

References


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