Neuronal maintenance via a neuron-specific degradation pathway

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Background and Hypothesis

Background: Neurons can survive for decades via cell maintenance. One aspect of cell maintenance is the general membrane protein endolysosomal degradation pathway. The Rab7 GTPase plays an integral role in that pathway, with *rab7* knockout mutantions resulting in neurodegeneration in adult *Drosophila melanogaster* fruit flies. Recently, components of a neuron-specific membrane protein endolysosomal degradation pathway were discovered. Two such components include the neuronal vesicle ATPase component V100 and the synaptic vesicle protein neuronal Synaptobrevin (n-Syb). The knockout of either of the genes encoding these proteins leads to neurodegeneration in adult *Drosophila*. Thus, the neuron-specific degradation pathway also is essential for the maintenance of neurons in adult *Drosophila*.

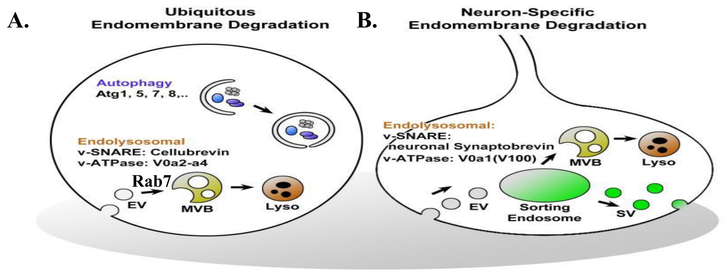

Figure 1. Overview of protein degradation. A) In ubiquitous endomembrane degradation, the Rab proteins provide specificity for intracellular compartments. The Rab proteins, when bound to GTP, attach to proteins called Rab effectors and enable vesicle fusion. The Rab7 protein is specific to late endosomes and is required for the appropriate sorting of vesicles. B) In neuron-specific degradation, the n-Syb protein is a SNARE protein that enables vesicle fusion and probably allows for the fusion of vesicles that contain degradative machinery with to-be-degraded vesicles. The V100 v-ATPase acidifies downstream degradative compartments like lysosomes and autophagosomes, and also probably enables fusion of synaptic vesicles with endosomes.

Hypothesis: Neurons have an increased or specialized need for endolysosomal degradation in comparison to other cells. While the existence of the neuron-specific degradation pathway is established, it is not specifically known what this pathway does, nor how it interacts with the general protein degradation pathway. This research aims to fill this gap in knowledge. Such research may be salient because the misregulation of protein degradation in neurons can lead to neurodegenerative diseases like dementia.

Methods

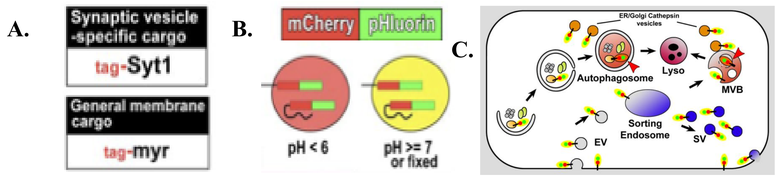

Figure 2. Visualizing membrane protein degradation. A) Synaptotagmin1 represents neuron-specific cargo, and a myristoylated protein represents general membrane cargo. B) The mCherry-pHluorin (mCh-phL-Syt1) tag that is sensitive to acidification. C) The mCherry-pHluorin tag progressing through the endolysosomal degradation pathway.

1. We chose a myristoylated protein (myr) to represent general proteins found in every cell, and Synaptotagmin1 (Syt1) to represent neuron-specific cargo (Figure 2A). The acidification-sensitive tag mCherry-pHluorin causes a change in color with a decrease in pH; it was placed on Syt1 and myr to visualize acidification and degradation of the two proteins (Figure 3B and 3C).

Methods, cont.

2. We generated *Drosophila* lines to compare acidification and degradation of Syt1 and myr in wild-type versus the following three mutants: *rab7* mutants to disrupt the general protein degradation pathway, *v100* to disrupt the neuron-specific protein degradation pathway, and *synaptobrevin* also to disrupt the neuron-specific pathway.

4. We performed live imaging to visualize acidification and protein degradation at synaptic terminals. Brains of *Drosophila* pupae from each cross were dissected, mounted onto Petri dishes, and surrounded with a culture medium to be kept alive. A resonant confocal microscope was used to observe the lamina, a layer of neurons between the eye and the brain where photoreceptor synaptic terminals are found. At the lamina, we recorded 30-minute videos showing changes in fluorescence representing protein degradation (Figure 4).

Figure 3. Schema of live imaging technique of *Drosophila* brains.

Results

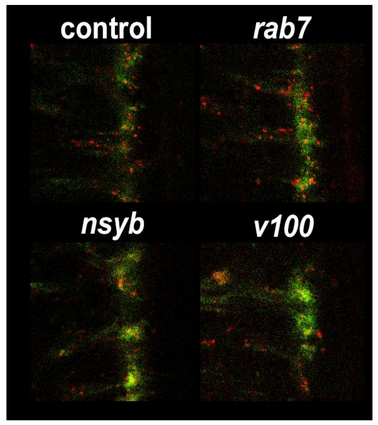

Figure 4. mCherry-pHluorin-Syt1 expression at synaptic terminals. Acidification of Syt1 at synapses may be impaired in *nsyb* and *v100* mutants, but not in *rab7* mutants. Fewer acidified compartments (red-only) are observed at the synaptic terminals of *nsyb* and *v100* mutants compared to the control. In contrast, the number of acidified compartments in *rab7* mutants looks similar to that in controls. Neuron-specific degradation pathway may be responsible for Syt1 degradation. Sample size of 5 per genotype.

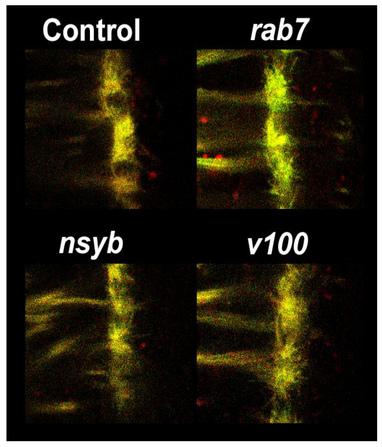

Figure 5. mCherry-pHluorin-myr expression at synaptic terminals. Acidification of the myristoylated protein does not appear to be affected in any of the mutants. The number of acidified compartments appears similar regardless of genotype. Quantitative analysis is needed. Sample size of 5 per genotype.

Conclusions

Our hypothesis stated that neurons require increased or specialized need for endolysosomal degradation, especially at synapses. We tested our hypothesis by visualizing endolysosomal degradation of neuron-specific membrane proteins and general membrane proteins in the context of mutations that specifically disrupt either the general membrane protein degradation pathway or the neuron-specific membrane protein degradation pathway. There are several potential relationships between general protein degradation and neuron-specific degradation:

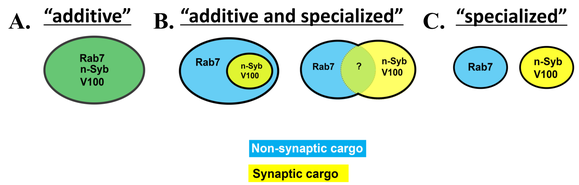

Figure 6. Three possible relationships between the general protein degradation pathway (represented by the Rab7 protein) and the neuron-specific protein degradation pathway (represented by the n-Syb and V100 proteins). A) In an additive scenario, general protein degradation and neuron-specific degradation work together to degrade both non-synaptic and synaptic cargo. B) In a mixed scenario, overlap exists between the degradation pathways. For example, the general degradation pathway may be essential to all degradation, while the neuron-specific pathway may only be essential for neuron-specific membrane protein. Or, each pathway may handle certain exclusive cargos, and both may contribute to certain "common" cargos. C) In a specialized scenario, the general degradation pathway degrades only non-synaptic cargo, and the neuron-specific pathway degrades only synaptic cargo.

Tentative conclusions:
1. The synaptic vesicle protein Synaptotagmin1 is specifically degraded by the neuron-specific endolysosomal degradation pathway.
2. The general pathway of protein degradation occurs at synapses, but has no specificity for synaptic vesicle cargo.

Future Directions

1. Quantitative analyses of acidification and degradation of Syt1 and myr.
2. Tag other SNARE proteins, such as syntaxin, to observe the degradation dynamics of other synaptic proteins.
3. Identify the acidified compartments. Are they autophagosomes? If not, what are they?
4. Determine if the degradation defects in the *v100* mutants are due to the loss of function of acidification in the V100 protein, or due to the loss of function in the V100 protein regarding synaptic vesicle fusion.

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