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Neuronal synaptobrevin Promotes Longevity in Drosophila Photoreceptors

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Abstract

Neurons have unique challenges relative to other cell types. Unlike most other cells, neurons must remain healthy and functional throughout the lifespan of an animal. Premature neuronal loss underlies many age-related neurodegenerative diseases, including Alzheimer and Parkinson Diseases. Despite previous research aimed at understanding the mechanisms of age-related neurodegenerative diseases, little is known about the mechanisms that allow neurons to remain functional for the lifetime of a healthy animal. Understanding these cellular and biochemical processes is essential to promote healthful aging and reduce the severity of neurodegenerative disease. Here we discuss our recent identification of neuron-specific proteins that regulate endosome fusion events and the role of endosomes in maintaining healthy neurons.

Keywords

endosome, nerodegeneration, membrane fusion, synaptobrevin, autophagy

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Neuronal synaptobrevin promotes longevity in Drosophila photoreceptors

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Neurons have unique challenges relative to other cell types. Unlike most other cells, neurons must remain healthy and functional throughout the lifespan of an animal. Premature neuronal loss underlies many age-related neurodegenerative diseases, including Alzheimer and Parkinson diseases. Despite previous research aimed at understanding the mechanisms of age-related neurodegenerative diseases, little is known about the mechanisms that allow neurons to remain functional for the lifetime of a healthy animal. Understanding these cellular and biochemical processes is essential to promote healthful aging and reduce the severity of neurodegenerative disease. Here we discuss our recent identification of neuron-specific proteins that regulate endosome fusion events and the role of endosomes in maintaining healthy neurons.

Keywords: endosome, neurodegeneration, membrane fusion, synaptobrevin, autophagy

Abbreviations: AD, Alzheimer disease; HD, Huntington disease; PD, Parkinson disease; *n-syb*, *neuronal synaptobrevin*; *syx1*, *syntaxin1*

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Unlike most cells of multicellular organisms, neurons cannot be replenished. A particular neuron's network of multicellular connections cannot be easily replicated by a new cell. Since neuronal death represents a permanent loss of function for an animal, neurons have been specialized to promote their health and functionality for as long as the organism survives. We call this characteristic neuronal longevity. Neurons actively resist and repair cellular damage, and dysfunction of these processes leads to neuronal degeneration.[1]

One process which promotes neuronal longevity is macroautophagy, herein referred to as autophagy.[2] Autophagy is the process by which cells degrade their own cytoplasm and organelles, either in response to starvation or to remove defective and long-lived components.[3,4] Autophagy begins with the formation of an phagophore, which encircles the material to be degraded and then fuses with itself to form a double-membraned autophagosome. Autophagosomes fuse with endosomes and lysosomes to acquire the proteases and acidification machinery required for degradation. As the autophagosome becomes acidified, it matures into an autolysosome and completes the degradation of its contents.

Multiple lines of evidence have shown that autophagy promotes neuronal longevity. Inhibition of autophagy causes neurons to degenerate in both flies and mice.[5-7] Interestingly, emerging evidence has demonstrated accumulation of defective autophagosomes and undegraded protein aggregates in many neurodegenerative diseases, including Alzheimer disease (AD), Huntington disease (HD), and Parkinson disease (PD). Moreover, treatment of mouse models of AD, HD, and PD with rapamycin, which induces high levels of autophagy, results in the clearance of aggregates and the reduction of disease symptoms.[8] Rapamycin treatment also rescues neurodegeneration in in vivo mouse models of progeria and ischemia, and in vitro models of oxygen or glucose deprivation.[9,10] Thus, autophagy keeps healthy neurons from degenerating and protects neurons from damage and disease.

Autophagy occurs in all cells, but is enhanced in neurons to promote neuronal longevity. Neurons have higher autophagic flux than other cells, meaning that autophagosomes mature and degrade their

contents faster in neurons than in other cells.[11] Two recent reports have found constitutive formation and retrograde transport of autophagic structures down the axons of live neurons.[12,13] Photobleaching experiments demonstrated that autophagosomes are formed in terminals but not axons.[13] However, these autophagosomes are not acidified in terminals, but become acidified during retrograde transport.[13] These data suggest that autophagosomes fuse with endosomes or lysosomes before entering the axon and mature as they move toward the soma.

Such neuron-specific regulation of autophagy requires the presence of neuron-specific regulatory factors. We recently identified *neuronal synaptobrevin* (*n-syb*) as such a factor in *Drosophila melanogaster*.[14] *N-syb*, known as Synaptobrevin or VAMP2 in mice and humans, is a SNARE protein that has long been known to mediate the fusion of synaptic vesicles with the presynaptic membrane to allow neurotransmitter release.[15] *N-syb* binds the SNAREs *syntaxin1* (*Syx1*) and *SNAP-25* on the presynaptic membrane to cause synaptic vesicle fusion. Loss of *n-syb* function through mutation or cleavage by tetanus toxin blocks synaptic vesicle fusion and neurotransmission. We found that *n-syb* mutant photoreceptor neurons degenerate over time.[14] Over five weeks, more than half of the photoreceptors in *n-syb* eyes degenerated. This degeneration was due to loss of *n-syb* function, as photoreceptor loss was rescued by the expression of an *n-syb* cDNA. Other mutations that block synaptic vesicle fusion or neurotransmission did not cause degeneration, indicating that degeneration is due to an unappreciated function of *n-syb*.

To identify this novel function, we first characterized the subcellular localization of *n-syb* protein. In the nerve terminal, *n-Syb* not only colocalized with synaptic vesicle markers but also with early endosome markers *Rab5* and *Syx7*. In *n-syb* mutant neurons, endosome markers accumulated up to four times wild-type levels. *N-syb* nerve terminals had high levels of proteins endocytosed from the plasma membrane. In fact, *n-syb* terminals swelled due to the amount of material they accumulated. Ultrastructural analysis showed that *n-syb* terminals were packed with small vesicles and autophagosomes. Importantly, autophagosomes were present at much higher levels than typically seen in neurons, and many already contained electron-dense material, indicating premature acidification and initiation of protein degradation. We concluded that *n-Syb* has a previously unappreciated role regulating endosomes and autophagosomes.

Since *n-syb* did not colocalize with autophagosomes, the autophagosome defects in *n-syb* neurons were likely a secondary effect of *n-syb*'s endosomal function. Autophagosomes fuse with endosomes or lysosomes to mature, so a defect in endosomal function can lead to non-maturing or slowly maturing autophagosomes. To assay endosomal function, we measured the maturation of the protease Cathepsin L. Cathepsins are synthesized as pro-proteins with an inhibitory domain blocking protease function. Pro-Cathepsins are transported from the Golgi apparatus to early endosomes, which then mature into late endosomes and lysosomes. As endosomes mature and become acidic, the Cathepsin pro-domains are cleaved off to activate protease function.[16] *N-syb* neurons accumulate high levels of pro- and mature-Cathepsin L. This data suggest that *n-syb* neurons accumulate early, non-acidified, endosomal compartments as well as maturing, acidified autophagosomes. We also found that pro-Cathepsin L accumulated before mature Cathepsin L during pupal development, indicating that endosomal dysfunction preceded autophagosome accumulation.

N-syb is not the only neuron-specific gene that regulates the endolysosomal system. Our previous work showed that *v100*, a neuron-specific subunit of the vacuolar ATP-ase, is required for the proper maturation of endosomes and autophagosomes in neurons.[17] The vacuolar ATP-ase acidifies endocytic organelles, but *v100* has an acidification-independent function regulating SNARE-mediated membrane fusion. *V100* localizes to synaptic vesicles and endosomes, regulating membrane fusion through direct interaction with *Syx1* on synaptic vesicles and *Syx7* on early endosomes.[17,18] Neurons mutant for *v100* accumulate synaptic vesicles, endosomes, and autophagosomes in a manner similar to *n-syb* mutants and they also degenerate over time.

Further analysis strengthened the connection between *n-syb* and *v100*. *N-syb* photoreceptors failed to localize *v100* to synapses. Moreover, overexpression of a *v100* cDNA in *n-syb* photoreceptors significantly rescued photoreceptor degeneration. Notably, a *v100* cDNA carrying a mutation that blocks its acidification function in the V-ATPase still rescued degeneration as well as the wild-type *v100* cDNA. Therefore, our work implicates both synaptobrevins and syntaxins in regulating endosome fusion to promote neuronal longevity.

SNAP-25, the third SNARE required for membrane fusion, may also protect against neurodegeneration. *CSP*α is a neuron-specific molecular chaperone whose loss causes neurodegeneration in flies and mice.[19] A recent study found that degeneration in murine CSPα neurons is primarily caused by loss of functional SNAP-25.[20] Thus, three neuron-specific genes regulate membrane fusion*: n-syb, v100,* and *CSP*α (**Fig. 1A**). These genes are required for fusion of synaptic vesicles with the presynaptic membrane, and their loss causes degeneration with an accumulation of autophagosomes. We have now shown that *n-syb* and *v100* both have important roles in endosome function. An endosomal role for *CSP*α has not been investigated to our knowledge.

We propose that the following mechanism promotes neuronal longevity (**Fig. 1B**): autophagosomes constitutively form at nerve terminal to degrade old and damaged proteins. These autophagosomes then fuse with endosomes before entering the axon. Endosomal V-ATPases and proteases cause the maturation of these autophagosomes into autolysosomes as they move down the axon. Therefore, any mutation or treatment that interferes with endosome protease function blocks autophagosome transport and maturation. Autophagosome accumulation would induce neuronal stress, leading to cell death. Thus, regulation of endosomes by neuron-specific factors is essential for proper autophagy and neuronal longevity.

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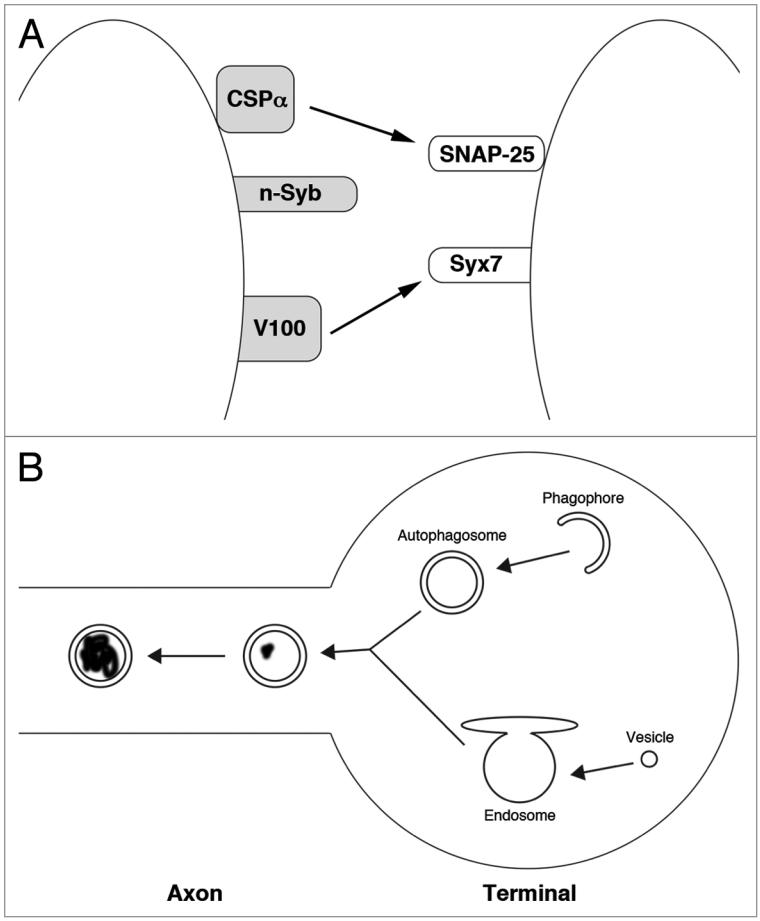

Figure 1. A model for endosomal regulation of neuronal longevity. **(A)** Neuron-specific factors regulate endosome-vesicle fusion. The SNARE proteins n-Syb, SNAP-25, and Syx7 directly control membrane fusion between neuronal endosomes and vesicles. CSPα is a chaperone that maintains the stability of SNAP-25. V100 binds to Syx7 and is required for fusion through a currently unclear mechanism. Neuron-specific proteins are gray. **(B)** Endosomes are required for the maturation of neuronal autophagosomes. Phagophores form and engulf cytoplasm, becoming autophagosomes, in nerve terminals. In order to mature, autophagosomes first fuse with endosomes, acquiring proteases and acidification machinery. Then, autophagosomes enter the axon and begin retrograde transport to the soma. As they move, autophagosomes begin to degrade their contents (degraded material is shown black material inside the autophagosome).

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