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Nibbling away at synaptic development

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Abstract

Beyond its role as a response to starvation, autophagy has been increasingly implicated as part of the normal mechanisms regulating growth and remodeling of various cells and tissues during development. In recently published work we demonstrate that autophagy promotes synaptic development of the Drosophila larval neuromuscular junction (NMJ). We find that autophagy acts by downregulating an E3 ubiquitin ligase, Highwire (Hiw), which limits NMJ growth via a MAPKKK pathway. A similar role for autophagy in the synaptic remodeling that occurs during learning and memory remains an intriguing possibility.

Keywords

neuromuscular junction; drosophila; synaptic development; ubiquitin-proteasome system; highwire; wallenda; MAPKKK

Normal neural function requires proper formation and growth of synapses to enable effective transmission of electrical information between two neurons or, in the case of an NMJ, between a motor neuron and a muscle fiber. Defects in synaptic development are associated with many human disorders including mental retardation, epilepsy and neurodegeneration. Structural remodeling of synapses along with functional changes in synaptic signaling is integral to molecular models of learning and memory. Yet detailed understanding of the cellular and molecular mechanisms governing synaptic growth and development is still lacking. Over the past decade, the NMJ of body wall muscles in Drosophila larvae has emerged as a leading model for studies of synaptic development because of its large size, easy accessibility and the extensive set of genetic and molecular tools that are available. Studies from a number of investigators have uncovered a variety of proteins and pathways that positively or negatively regulate larval NMJ growth, including cell adhesion molecules, cytoskeletal components, BMP signaling, Wnt signaling, and a MAPKKK pathway whose output is dependent on Hiw, which is thought to target the MAPKKK encoded by Wallenda (Wnd) for degradation via the ubiquitin-proteasome system. Loss of Hiw activates the pathway and results in the most striking NMJ overgrowth phenotype yet described, with extensively elongated synaptic branches and a 3- to 4-fold increase in the number of synaptic boutons—the sites of actual communication between pre- and postsynaptic cells.

We have now added an important new pathway to the list of those involved in synaptic growth control—autophagy. We find a consistent relationship between levels of autophagy and extent of NMJ growth. Autophagy mutants (*atg1*, *−2*, *−6* and *−18*) whose normal functions span the entire process of autophagy, all exhibit NMJ undergrowth with bouton numbers reduced by about half. Thus, a basal level of autophagy is required to promote normal NMJ development.

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Conversely, manipulations that trigger autophagy, such as Atg1 overexpression, result in NMJ overgrowth with bouton numbers increased by 2- to 3-fold. Moreover, overgrowth associated with Atg1 overexpression is suppressed by mutations in *atg18*, demonstrating that this overgrowth is due to elevated levels of autophagy rather than to some other downstream pathway. Tissue-specific rescue experiments indicate that the observed NMJ undergrowth and overgrowth depend primarily on pre-synaptic levels of autophagy.

Even more surprising than the discovery that autophagy has such strong effects on synaptic development in Drosophila is the mechanism through which it operates. The phenotypic appearance of the overgrown NMJs when autophagy is induced provided the first clues, as the pattern of overgrowth closely resembles that of *hiw* mutants, suggesting a possible convergence of these two degradative pathways. Multiple lines of evidence support this idea. We found that mutations that suppress NMJ overgrowth caused by loss of Hiw, including mutations of Wnd and expression of a dominant-negative mutation in the Jun Kinase encoded by *basket* (*bsk*), which is downstream of Wnd, also suppress Atg1-dependent NMJ overgrowth, indicating that autophagy converges with the Hiw pathway upstream of Wnd. Moreover, rather remarkably, the point of convergence appears to be Hiw itself. Not only is the level of Hiw reduced when autophagy is elevated but restoring lost Hiw by weak overexpression of hiw^{+} suppresses Atg1-dependent NMJ overgrowth. This result is of particular importance because it argues that Hiw is not only a target of autophagy, but it is also a target of primary functional importance with respect to NMJ development, because if NMJ overgrowth were influenced by autophagy-dependent decreases in the level of many different proteins, simply restoring Hiw should not rescue the phenotype.

Conversely, NMJ undergrowth caused by impaired autophagy can largely be explained by a corresponding increase in Hiw levels. Thus, loss of Hiw prevents NMJ undergrowth caused by *atg* mutations. In addition, moderate overexpression of Hiw causes mild NMJ undergrowth, which is further enhanced by removing one copy of $atg1^{+}$, -2^{+} or -6^{+}. Finally, Hiw levels are significantly increased when autophagy is disrupted. However, excess Hiw cannot fully explain NMJ undergrowth in *atg* mutants because the undergrowth phenotype associated with Hiw overexpression is less severe than that of *atg* mutants. Most likely, when autophagy is impaired, accumulation of additional negative regulators contributes to depressed NMJ growth.

These findings raise many additional questions: Is Hiw specifically degraded by autophagy or is it just the one protein among many synaptic proteins that are degraded whose levels are most critical for NMJ development? If Hiw is targeted specifically to autophagosomes, how does this occur? Recent studies have described other examples of specific substrates being targeted to autophagosomes but no general rules for this mechanism have yet emerged. Does control of NMJ growth by autophagy involve local regulation at presynaptic terminals or is it mediated by events taking place in the cell body? Local control affords the opportunity for developmental plasticity at individual synaptic branches without affecting all synaptic terminals of a particular neuron, which could be important for synapse-specific modifications related to learning and memory. Does autophagy play additional roles on the postsynaptic side to regulate NMJ development and function? Does autophagy have a similar role in regulating growth of synapses in the central nervous system? Is synaptic development in vertebrates also regulated by autophagy? What is the physiological role of autophagy in regulating synaptic growth—is it responding to diet, crowding, temperature, or other environmental cues? Because autophagy is perfectly positioned to link a variety of environmental conditions with synaptic growth and plasticity, could it play a role in synaptic remodeling during learning and memory? Clearly, there is much of interest left to nibble on.

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