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A specific ATG-4 isoform is required for autophagic maturation and clearance in *C. elegans* neurons

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Abstract

The mechanisms that coordinate transport and clearance of synaptic autophagosomes are poorly understood. We performed forward genetic screens in *C. elegans* for mutants that abnormally accumulate autophagosomes in neurons, and identified ATG-4.2, a cysteine protease previously thought to be largely redundant with ATG-4.1. We find that atg-4.2 mutant animals, but not atg-4.1 mutant animals, have specific defects in autophagic maturation, resulting in accumulated autophagic vacuoles containing synaptic materials in the neuronal cell bodies. Defects in autophagic clearance are enhanced in animals also mutant for *unc-16/Mapk8ip3/Jip3*, which we find regulates retrograde transport of autophagosomes. This study demonstrates that ATG-4 isoforms can be specialized to perform distinct functions, from autophagosome biogenesis (through ATG-4.1, and also ATG-4.2) to autophagic vacuole clearance (primarily through ATG-4.2). Our study also highlights how dysfunction in distinct steps of the macroautophagy/autophagy process (transport and clearance) can result in enhanced autophagosome accumulation as seen in neurodegenerative diseases.

Article

Autophagy is a cellular degradation process, which is conserved from yeast to mammals, and which also occurs in specialized cell types, such as neurons. In the nervous system, autophagy at the synapse has been correlated with synaptic activity. In our study we demonstrate that in *vivo* in *C. elegans* the number of synaptic autophagosomes predictably varies depending on the firing state of the neuron, which we can manipulate by altering physiological stimuli that promote neuronal responses, by genetically inhibiting synaptic transmission or by chemically stimulating. Later Atg4s cleave Atg8s to remove them from the autophagosome to allow for autophagic maturation and degradation (delipidation). In yeast there is one Atg4 protein that performs both of these functions, whereas in mammals there are 4 ATG4s with different affinities for the 7 LC3/GABARAP family members. Previous biochemical studies of the mammalian isoforms have shown that different isoforms have different affinities for priming or delipidation activity. These findings raise the possibility that different isoforms may act in different steps (priming or delipidation) of the autophagy process (Figure 1).
In *C. elegans*, there are 2 Atg4 enzymes, ATG-4.1 and ATG-4.2. We therefore examined whether *atg-4.1* and *atg-4.2* acted similarly. We found that *atg-4.1* mutant animals do not display autophagic accumulation, which was surprising because at the time ATG-4.2 was thought to be redundant with ATG-4.1. To complement our *in vivo* studies, we performed electron microscopy and found that *atg-4.2*, but not *atg-4.1*, mutant animals accumulate multilamellar structures in *C. elegans* neurons.

Are these 2 Atg4 proteases performing different functions *in vivo*? We examined *atg-4.1*; *atg-4.2* double mutant animals and observed a severe loss of LGG-1 puncta, suggesting that *atg-4.1* and *atg-4.2* are redundant for LGG-1 priming. However, our *in vivo* genetic data indicate that ATG-4.2 might be specialized for delipidation. We purified ATG-4.2 and tested its cleavage efficiency toward soluble LGG-1 (priming) versus lipidated LGG-1 (delipidation), and found that ATG-4.2 was more efficient at cleaving the lipidated substrate.

Together these data are consistent with a model where ATG-4.1 and ATG-4.2 are partially redundant in priming, but ATG-4.2 is specialized for delipidation of LGG-1/2.

Why do autophagosomes accumulate in *atg-4.2* mutant animals? We examined if the *atg-4.2* mutant defects in delipidation of LGG-1/2 also affect the autophagic maturation process. Using a tandem label strategy to monitor LGG-1 acidification and a lysosome marker, LAAT-1, to monitor autolysosome formation, we determined that autophagosomes in *atg-4.2* mutant animals fail to undergo maturation and fusion with lysosomes. We then examined if loss of ATG-4.2 function also affects accumulation of autophagosome synaptic cargos. We monitored cargo acidification by fusing the tandem label to the synaptic protein SYD-1 and found that *atg-4.2* mutant animals accumulate non-acidified synaptic cargo in the neuronal soma. These results suggest that autophagosomes with synaptic cargos are transported to the cell body where their maturation and clearance is then dependent on delipidation of LGG-1/2 by ATG-4.2. Furthermore, our findings reveal that LGG-1/2 needs to be removed from the autophagic membrane for autophagosome maturation and cargo degradation.

Is there a benefit to specialized functions for the ATG-4 isoforms *in vivo*? We speculate that this specialization might provide targets for regulation. For example, regulated inhibition of ATG-4.2 would, in theory, pause autophagic progression without affecting new biogenesis (as priming continues due to partial redundancy with ATG-4.1). Understanding the specialized functions of these proteases could provide important pharmacological targets for regulating specific steps of autophagy, and allow for precise control of autophagy flux in distinct cell types and diseases.

**Disclosure statement**

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