

Addendum

Drosophila Atg7

Required for stress resistance, longevity and neuronal homeostasis, but not for metamorphosis

Gábor Juhász^{1,2} and Thomas P. Neufeld^{1,*}

¹University of Minnesota; Department of Genetics, Cell Biology and Development; Minneapolis, Minnesota USA; ²Eötvös Loránd University; Department of Anatomy, Cell and Developmental Biology; Budapest, Hungary

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Autophagy, the lysosomal degradation and recycling of self material, has been implicated in a number of developmental and pathological conditions including aging, cancer, neurodegeneration, and insect metamorphosis. Surprisingly, *Atg7* mutant flies are able to complete metamorphosis with only a slight delay, despite strongly reduced autophagy levels. Similarly, developmental elimination of the larval midgut proceeds with normal morphology, suggesting that animals can compensate for reduced autophagy during development. *Atg7* mutant adults are hypersensitive to starvation and oxidative stress, live shorter, and accumulate ubiquitin-positive aggregates in the brain that lead to a progressive decline of neuronal function and cell death. These results suggest that in *Drosophila*, normal levels of autophagy may play a more important role in the homeostasis of certain terminally differentiated cells and stress survival than during development.

Autophagy is a major catabolic process in eukaryotic cells, capable of degrading all types of biological macromolecules in the lysosomal compartment. Autophagy is greatly upregulated in response to starvation, thereby providing synthetic processes with building blocks and energy in the absence of exogenous nutrients to promote survival. In multicellular animals like flies and mice, this starvation response is tightly coordinated at the organismal level, with various tissues responding differently (ref. 1 and our unpublished data).

Examples of developmentally programmed natural starvation periods are found in newborn mice when placental nutrition is interrupted, until milk feeding provides new nutrients,² and in holometabolic insects such as *Drosophila* during molting and metamorphosis. Indeed, a very strong induction of autophagy can be observed in most polyploid larval tissues immediately preceding and during metamorphosis.³ This developmental autophagy is thought to

serve a similar function as in the case of starvation: pupae are immobile and do not eat for 5 days, so autophagy of larval tissues has to support the proliferation and differentiation of diploid imaginal cells that give rise to the adult fly.

Given these considerations, it seemed likely that *Drosophila* mutants of nonredundant autophagy (*Atg*) genes would be lethal. Indeed, *Atg1* null mutants were previously shown to die during the late pupal period.⁴ Surprisingly, however, we were unable to isolate *Atg7*- or *Atg3*-specific mutations using lethality-based selection scenarios.^{5,6} In the case of *Atg7*, we confirmed that this was due to the fact that *Atg7* is not an essential gene: *Atg7* mutant flies are fully viable and fertile.⁵

How can these animals complete metamorphosis normally without functional Atg7 protein? A likely explanation is that Atg mutations drastically reduce autophagy, but do not eliminate it completely. In the case of *Atg7* mutants, morphometric analysis of electron micrographs revealed an 85–95% reduction in the total area of autophagic structures. The remaining 5–15% residual autophagy may be sufficient to maintain metamorphosis, with extra time needed to compensate for the reduction, as was found for *Atg7*. Thus, while the results of this study indicate that the large burst of autophagy observed during pupariation (the larval-prepupal transition) is not essential for development, some minimal level of autophagy may be necessary for normal metamorphosis. This interpretation further implies that *Atg* genes with lethal mutant phenotypes, such as *Atg1*, likely have additional roles in other essential developmental processes. Further studies, most importantly generation and full characterization of additional *Atg* null mutations, will be necessary to answer these questions.

Using our viable mutants, we established the role of *Atg7* in a variety of adult functions. As expected, these flies are hypersensitive to starvation and oxidative stress, conditions when autophagy is beneficial to survive nutrient limitation or clear up damaged macromolecules and organelles. In addition, *Atg7* mutants recover more slowly from a 10-minute CO₂ anesthesia or a chill coma (exposed to 4°C for 4 hours) (Fig. 1A and B), which may indicate neuronal defects. In line with this, adult *Atg7*-deficient flies show an age-dependent loss of climbing ability, an established measure of neuronal function. Electron microscopy of mutant brains reveals the progressive accumulation of ubiquitinated protein aggregates, resembling the phenotype of brain-specific *Atg5* and *Atg7* knockout mice.^{7,8}

*Correspondence to: Thomas P. Neufeld; University of Minnesota; Department of Genetics, Cell Biology and Development; 6-160 Jackson Hall; 321 Church St.; S.E. Minneapolis, Minnesota 55455 USA; Email: neufeld@ahc.umn.edu

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These inclusion bodies have no limiting membrane, indicating that they form in the cytoplasm. Membrane fragments can occasionally be found inside the aggregates, potentially representing organelles that get randomly trapped in the forming inclusion body (Fig. 1C).

These results suggest that impaired protein turnover and neuronal homeostasis are responsible for the neurodegeneration and short lifespan of *Atg7* mutants, due to impaired basal autophagy. Interestingly, hypomorphic *Atg8a^{EP362}* mutants that show no defects in starvation-induced autophagy⁴ also accumulate aggregates in neurons, often surrounded by a membrane.⁹ It is tempting to speculate that such inclusion bodies form due to the partial reduction in basal autophagy in these mutants, with some fraction of them degraded by autophagy.

Caution has to be exercised, though, as basal autophagy has been demonstrated in a number of tissues including the liver and pancreas, but has not yet been observed directly in healthy adult mouse (or fly) brains.¹ Therefore, although unlikely, we cannot completely exclude the possibility of an autophagy-independent function of *Atg* genes that may be involved in aggregate formation. Indeed, mammalian orthologs of *Atg8* include not only LC3, the bona fide autophagy protein, but also GABARAP, a modulator of gamma-aminobutyric acid receptor trafficking, and GATE-16, a Golgi-associated ATPase enhancer. Each of these proteins is activated by lipidation through the same molecular mechanism.¹⁰ Future work will shed more light on the so-far elusive process of basal autophagy in neurons.

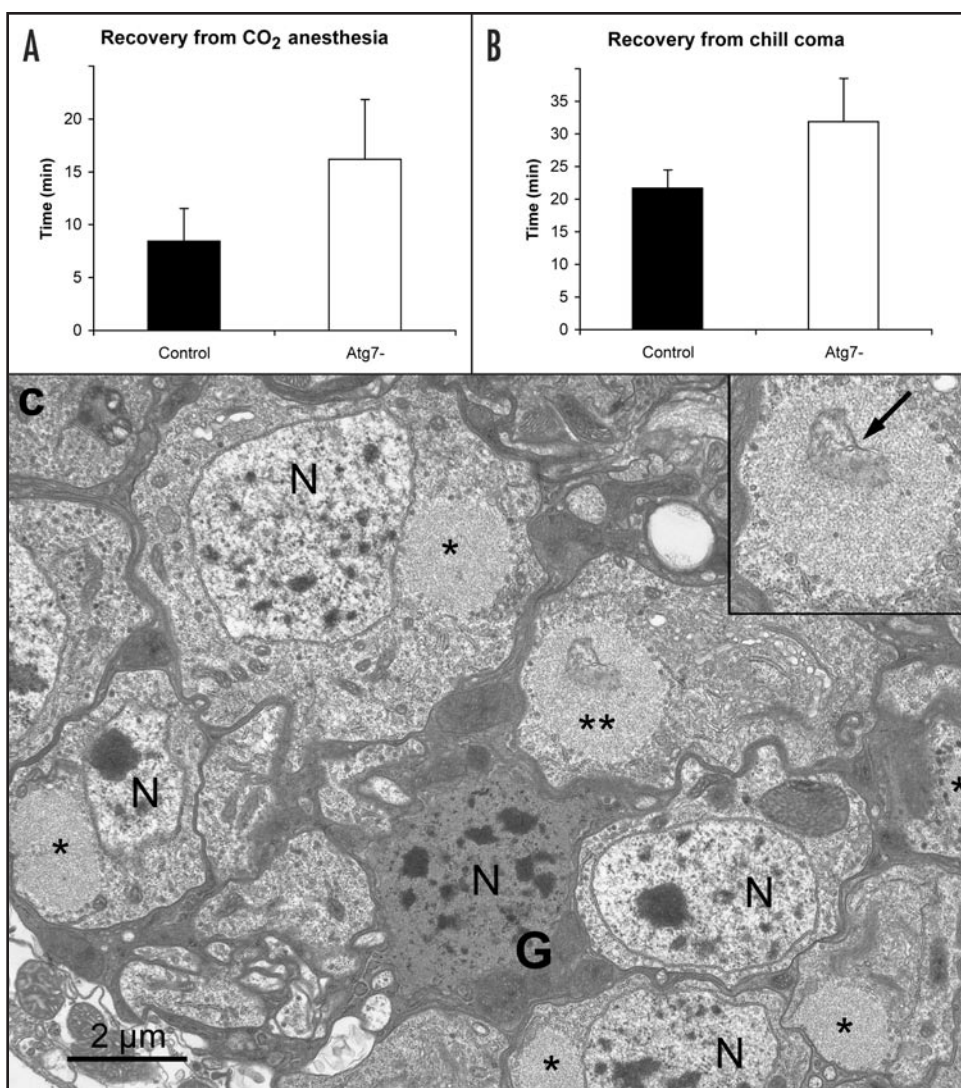


Figure 1. *Atg7* mutants recover more slowly from CO₂ anesthesia (a) and chill coma (b). Student's T-test was used to determine significance; *p* values < 10⁻¹⁵. Panel c shows an electron micrograph of a 30-day old *Atg7* mutant brain. Note the widespread accumulation of inclusion bodies (asterisks) in neurons. G indicates a glial cell that isolates surrounding neurons by its thin cytoplasmic processes, recognized by its more electron dense cytoplasm and nucleus. Nuclei are marked by N. The inset shows enlargement of a protein aggregate (marked by double asterisks) that contains membrane elements, most likely remnants of a mitochondrion (arrow).

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