Tissue-specific requirement of the autophagy gene *atg-18* in controlling *C.elegans* dauer morphogenesis, fat metabolism and adult longevity

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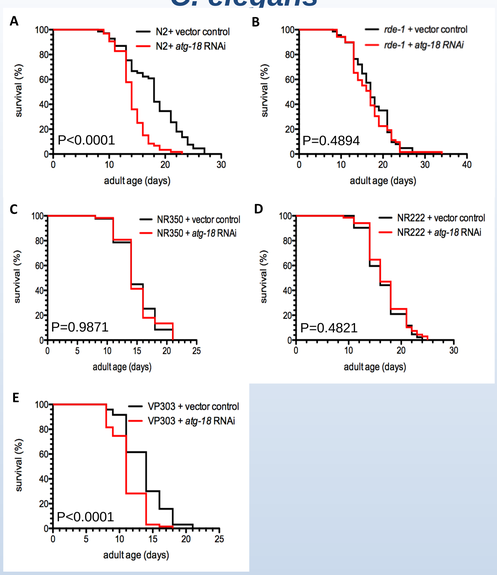
Introduction

The conserved insulin growth factor (IGF) signaling pathway is one of the major regulators of lifespan in many species including *C. elegans*. In *C. elegans* the insulin/IGF-like receptor is encoded by the *daf-2* gene, mutations in which result in lifespan extension, fat accumulation and dauer formation. The *daf-2* activity in the nervous system controls these phenotypes cell non-autonomously. Interestingly, the longevity phenotype of *daf-2* mutant worms is dependent on macroautophagy (hereafter autophagy). Autophagy is a highly conserved lysosomal degradation pathway involved in the removal of long-lived proteins and cytoplasmic organelles. During autophagy, cellular components are sequestered into the double-membrane autophagosomes and delivered to lysosomes for degradation. Increasing evidence has emerged that the autophagy process is a central regulator of lifespan that is required for the effects of DAF-2 signaling, dietary restriction and some mitochondrial mutations on *C. elegans* longevity. It is unknown however whether autophagy activity in every tissue or in a single tissue mediates the influence of these longevity signals. To address this question, we examined the tissue requirement of autophagy gene *atg-18* for the lifespan of wild type animals and the *daf-2* mutant. We discovered that neurons and intestinal cells are two key tissues where *atg-18* mediates the effect of DAF-2 insulin-like signaling on lifespan, fat accumulation and dauer morphogenesis, suggesting autophagy acts cell non-autonomously in controlling *C. elegans* dauer formation, fat metabolism and adult longevity.

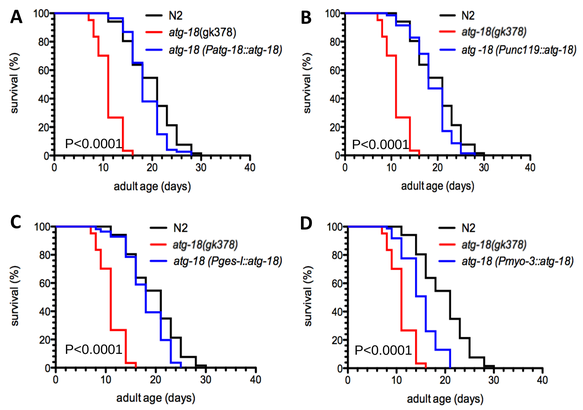
Results

Figure 1. Intestine-specific RNAi knock down of *atg-18* decreases the lifespan of *C. elegans*



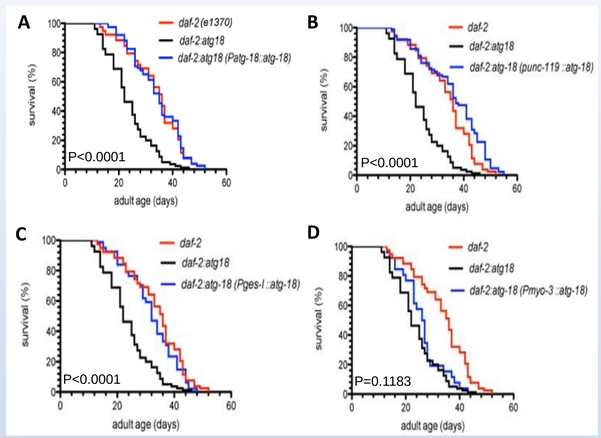
atg-18 RNAi treatment decreases the lifespan of N2 worms (A) but fails to decrease the lifespan of RNAi – defective *rde-1* mutant worms (B), and the lifespans of animals only sensitive to RNAi in body wall muscles (NR350) and in hypodermal cells (NR222) (C and D). The intestine – specific RNAi sensitive strain (VP303) shows a decreased lifespan due to the inhibition of *atg-18* gene (E).

Figure 2. Expression of *atg-18* in neurons or intestine restores the lifespan of *atg-18* mutants to wild type level



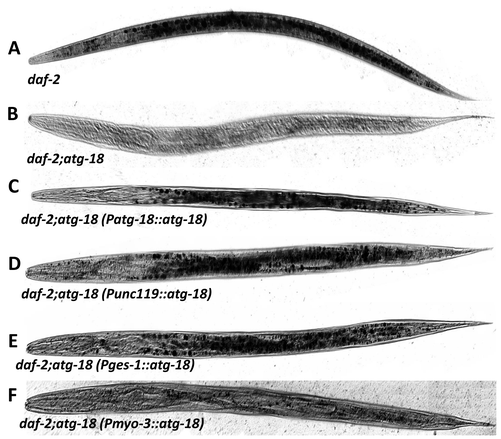
Natively expressed *atg-18* (*Patg-18::atg-18*) or expression of *atg-18* in either the neurons (*Punc-119::atg-18*) or intestine (*Pges-1::atg-18*) alone rescues the decreased lifespan of *atg-18* mutant (A, B and C) while rescuing *atg-18* in the body wall muscles (*Pmyo-3::atg-18*) only partially restores the lifespan (D).

Figure 3. Neuronal and intestinal expression of *atg-18* is essential for the longevity phenotype of *daf-2* mutants



Rescue of *atg-18* in either the neurons or intestine completely restores the longevity phenotype of the *daf-2* mutants (B and C). Expression of *atg-18* in the body wall muscles fails to restore the longevity phenotype (D).

Figure 4. Tissue-specific requirement of *atg-18* for the fat accumulation in *daf-2* mutants



Fat accumulation detected by Sudan black staining in dauer larva with indicated genotypes. Fat accumulation in *daf-2* mutants (A) is suppressed by *atg-18* mutations (B). Expression of *atg-18* driven by its native promoter, neuron-specific or intestine-specific promoters restores the fat accumulation in *daf-2;atg-18* mutants (C, D and E). Expression of *atg-18* in body wall muscles has no obvious effect on fat accumulation in *daf-2;atg-18* mutants (F).

Table 1. Tissue-specific requirement of *atg-18* for the *daf-2* signaling – mediated dauer morphogenesis measured by 1% SDS resistance

Strains	SDS resistance	
N2	0%	(0/50)
daf-2	97%	(105/108)
atg-18	0%	(0/45)
daf-2;atg-18	63%	(51/81)
daf-2;atg-18(Patg-18::atg-18)	96%	(52/54)
daf-2;atg-18(Punc-119::atg-18)	87%	(104/119)
daf-2;atg-18(Pges-1::atg-18)	92%	(49/53)
daf-2;atg-18(Pmyo-3::atg-18)	83%	(57/69)

The dauer formation was induced for all *daf-2* transgenic lines by incubation at 25°C. L3 larva of N2 and *atg-18* worms grown at 25°C were used as controls.

Conclusions

❖ In the wild type background the absence of autophagy in the *atg-18* mutant results in a large decrease in lifespan. When autophagy is rescued in either the neurons or intestine the lifespan of the *atg-18* worms is brought back to wild type levels. In addition, there is a partial restoration of lifespan seen in a body wall muscle rescue of autophagy. This shows that autophagy functions in a cell non-autonomous manner, with the neurons and intestine being the most important tissues where it acts. However, autophagy in other tissues may also play minor roles, such as in the body wall muscles.

❖ The longevity phenotype of the *daf-2* mutants is abolished when the autophagy gene *atg-18* is mutated. This longevity can be completely restored in *daf-2;atg-18* worms by only rescuing *atg-18* in either the neurons or the intestine suggesting that autophagy mediates the *daf-2* signaling-mediated lifespan extension cell non-autonomously.

❖ In addition to regulating the lifespan of *daf-2* mutants, autophagy also controls the fat accumulation. The high fat accumulation of *daf-2* mutant worms is not observed in the *daf-2;atg-18* double mutants. The fat accumulation is significantly restored in *daf-2;atg-18* mutants when *atg-18* is rescued in the neurons or intestine. However, expression of *atg-18* in the body wall muscles appears to have no obvious effect on fat accumulation.

❖ Autophagy is required for the dauer morphogenesis of *daf-2* mutant dauer larva. *daf-2;atg-18* mutant worms show only a 63% resistance to 1% SDS. Expression of *atg-18* in neurons, intestine or body wall muscles significantly but not fully restores the SDS resistance of *daf-2;atg-18* mutant dauer larva, suggesting expression of *atg-18* in all of these tissues may be required for completion of dauer morphogenesis in *daf-2* mutant dauer larva.

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