The Role of Mitophagy in the Longevity of *Caenorhabditis elegans*

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Introduction

Neurodegenerative diseases such as Parkinson's Disease (PD) affect more than a million Americans, costing millions of dollars in health care. It has been shown that oxidative stress plays a role in the pathogenesis of this disease. In an effort to look for possible protective mechanisms to combat this disease, we study autophagy. Autophagy ("self eating") is an evolutionary conserved lysosomal degradation pathway which has been shown to increase the survival of *Caenorhabditis elegans* during starvation[1, 2] and is essential for *C. elegans* normal life span. Interestingly, neuronal knockout of autophagy gene results in neurodegeneration in mice[3]. In this project we are investigating the role of mitophagy, the autophagy process to degrade mitochondria, in defense against oxidative stress. Mitochondria are the principal organelles within the cell in charge of providing all the required energy. As a byproduct Reactive Oxygen Species (ROS) are released harming the cell. We inhibit mitophagy by knockdown of *atg 18*, an essential gene in mitophagy, through the RNAi mechanism. *atg 18* RNAi treated worms are exposed to Paraquat (PQ) and the survival of worms is examined. PQ is a chemical which increases the ROS generation[4] and a structural analog of the toxin that induces Parkinson's disease[5] by degenerating dopaminergic neurons[6]. **We want to see if inhibition of mitophagy will make worms more susceptible to PQ, which will help us determine the role of mitophagy in protecting *C. elegans* against oxidative stress, perhaps also in humans.**

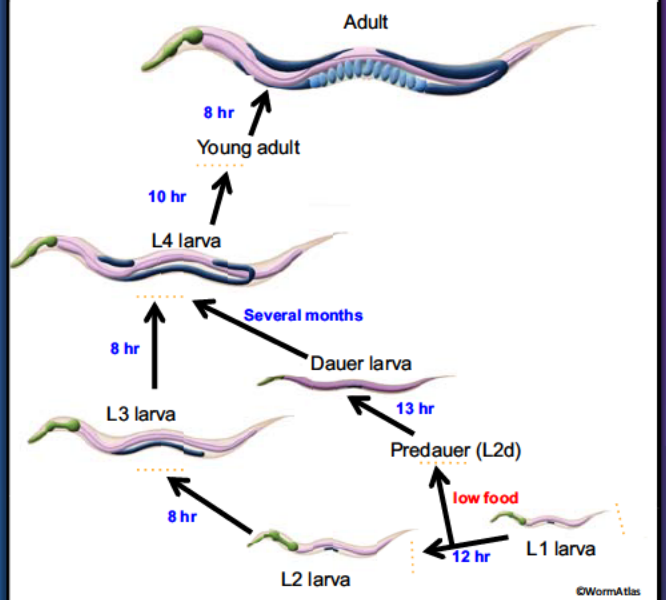

Figure 1 *C. elegans*, our model organism. This illustration depicts the 58-hour life cycle at an incubation temperature of 25° C. Lack of nutrients (starvation) will cause the worm to go into the dauer larval stage when its development and growth are arrested. When nutrients are introduced, *C. elegans* dauer larva can go back into their normal life cycle. We set up the experiment with worms at the L4 larval stage to not jeopardize development and to synchronize the worms.

MITOPHAGY

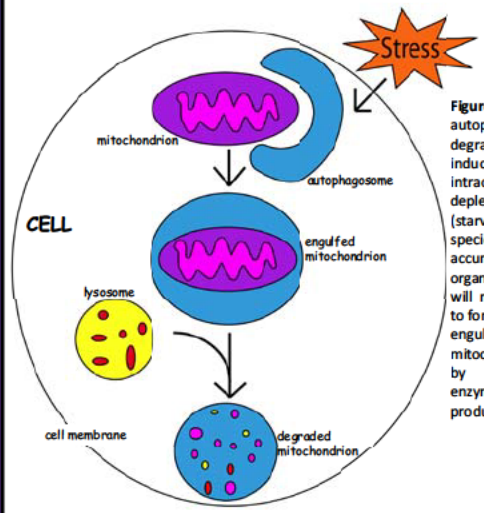

Figure 2 Mitophagy, the autophagy process to degrade mitochondria, is induced by extracellular and intracellular stresses such as depletion of nutrients (starvation), reactive oxygen species (ROS), or accumulation of damaged organelles. Autophagosome will merge with a lysosome to form autolysosome where engulfed cargo such as mitochondria are degraded by lysosomal hydrolytic enzymes and the breakdown products are reused.

Methods

Liquid Assay

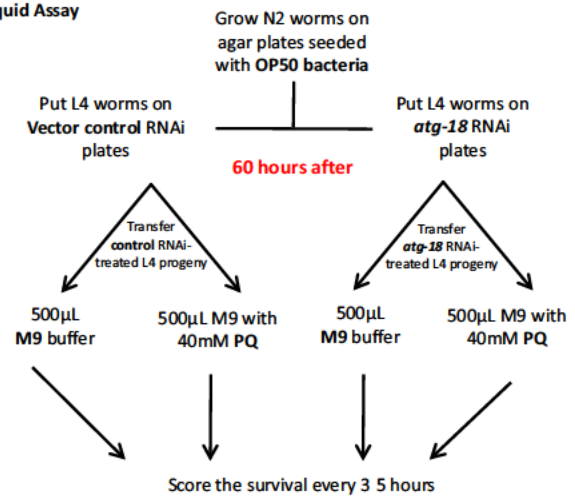

Figure 3 Liquid assay: multiple-step procedure where wild-type N2 worms are fed with either *atg-18* RNAi or vector control RNAi bacteria at 20° C . ***atg-18* RNAi** bacteria will silence ATG-18 protein. After 60 hours, the *atg-18* RNAi and control RNAi treated L4 progeny are put in the wells of depression slides. Each well holds a total of 500 µL of liquid. In two wells, which will be our control, 500µL of M9 buffer are added. In another 2 wells, 500 µL M9 buffer containing 40mM PQ are added. Twenty-two worms from each group were transferred to each well, respectively. At the end, we have 4 wells filled with either M9 buffer or 40mM PQ. The survival of the worms is then scored every 3 hours until all the worms in the PQ buffer are dead.

Results

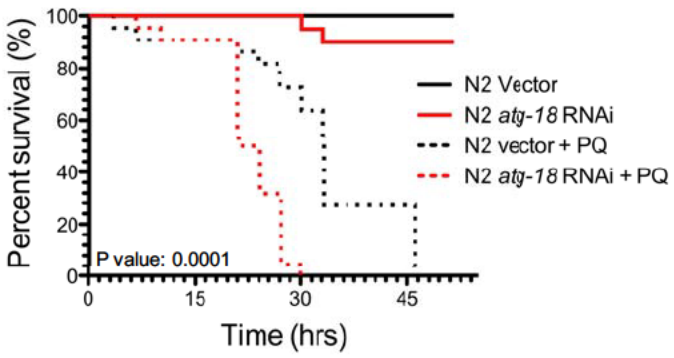

Figure 4 Survival of N2 adults in the presence of Paraquat at 20° C. As expected, the knockdown of autophagy gene *atg-18* increased the worms' susceptibility to oxidative stress induced by PQ. While *atg-18* RNAi had no obvious effect on N2 life span without PQ treatment, it significantly decreased the life span of PQ-treated worms. The mean life span was decreased by 33%. For maximum life span, vector RNAi-treated worms lived 46 hours when they were exposed to 40mM PQ, whereas the *atg*-18 RNAi-treated worms lived only 30 hours under the influence of the same concentration of PQ.

Conclusions

❖Paraquat decreases the lifespan of the worms since its presence generates ROS which cause oxidative damage to animals.

❖Knockdown of the mitophagy gene *atg 18* increases the worms' susceptibility to oxidative stress induced by paraquat.

❖The data support our hypothesis that mitophagy protects worms against oxidative stress. It could also be true in humans since the mitophagy process is highly conserved from *C. elegans* to humans. Thus, activation of mitophagy by pharmaceuticals may help fight against oxidative stress in humans, which is beneficial to Parkinson's disease patients.

References

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