Identification of *C. elegans* Ortholog of Spinster

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Introduction

Autophagy, an evolutionarily conserved lysosomal degradation pathway, is critical for cell survival under starvation. *Spinster* is a putative lysosomal efflux permease that has been linked to autophagy function in mammalian cells (1, 2) but not yet in a multicellular organism up to this point. Both autophagy and Spinster are involved in the pathogenesis of lysosomal storage diseases (3). Understanding how spinster influences autophagy can help to discover new therapeutic targets for these diseases. Based on sequence homology, there are four predicted Spinster orthologs in *C. elegans*. Deletion mutants for each *spinster* are available. In our preliminary studies, we measured the survival of one candidate *spinster* mutant (RB1702) and the wild type strain of *C. elegans* under starvation. The survival rate of RB1702 is significantly decreased compared to the control animals, suggesting Spinster is essential for survival during starvation possibly by influencing autophagy.

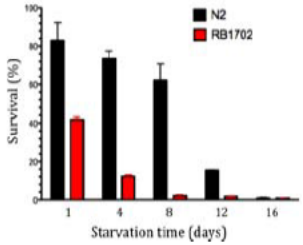

Figure 1. Survival of candidate *spinster* mutants RB1702 and wild type N2 worms after starvation.

In this present study, our goal was to identify the truly functional *C. elegans* Spinster by performing a starvation assay. We hoped to use *C. elegans spinster* mutants as a genetic model to understand the function of spinster and its role in autophagy in humans. We hypothesized that because autophagy is essential for *C. elegans* to survive under starvation conditions, which ever *spinster* mutant does not survive starvation is the most likely candidate for the *spinster* gene in *C. elegans*.

Method

Materials

1. Four *spinster* deletion mutants (*RB1702*, *RB1778*, *RB1986*, *RB1678*)
2. Wild type N2 worms.
3. *C. elegans* agar plates seeded with *E. coli OP50* bacteria (food plates).
4. M9 buffer lack of nutrients to simulate starvation conditions.
5. 15ml Conical vials
6. Glass Pipettes to transfer worms.
7. Refrigerated incubator for storage of worms set to 15 degrees Celsius.
8. Shaker

Procedures

1. Mutants and wild type worms underwent egg preparation to isolate their eggs. The eggs were kept in M9 buffer at room temperature for 24 hours.
2. After 24 hours, all the hatched worms arrested development at the first larval stage (L1) (synchronized). The L1 larvae were left in 3 mL of M9 Buffer solution (lack of nutrients) for different lengths of starvation time ranging from 1 day to 16 days after this point. Day 1 is 24 Hrs. after egg preparation, and so on. A total of Five Lengths of starvation (1, 4, 8, and 16 days of starvation) were measured.
3. At each starvation length, twenty micro liter aliquots or approximately 200 starved L1 larvae of each worm strain were plated onto 4 agar plates seed with *E. coli OP50* bacteria food.
4. After 72 hours from when the L1 larvae of each worm strain were plated to the corresponding days of starvation, the number of adult worms (survival from the starvation) were counted and averaged from its four plates. The number of adults that developed from Day 0 of starvation (meaning not starved) from each worm strain was used as the control and as the denominator to calculate the percentage of worms recovered after a length of starvation.

Results

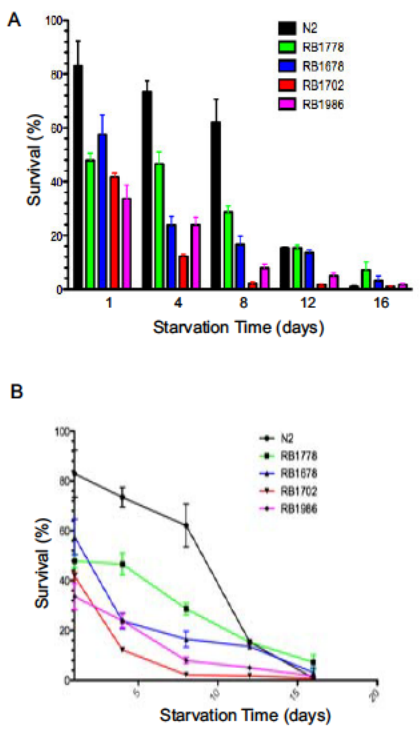

Figure 2. Survival of four candidate *spinster* mutants in the starvation assay. (A) Bar Graph and (B) Line Graph showing the percentage of worms recovered to develop into adults after each corresponding length of starvation. The survival rate of each examined candidate *spinster* mutant is significantly decreased compared to control wild type N2 animals ($p < 0.01$, *t* test) except for groups starved for 16 days.

Discussion

1. A null mutation in each candidate *spinster* gene significantly decreased the survival of mutants, suggesting these candidate genes are all required for *C. elegans* to survive under starvation conditions. Since Spinster is a putative lysosomal efflux permease, the data presented here raises one interesting possibility that each Spinster may transport one kind of metabolite out of lysosomes in *C. elegans*. Thus, mutations in any of them would decrease the survival of animals during starvation.
2. The *RB1702 spinster* mutant had the greatest rate of decline in survival than any of the other *spinster* mutants. This suggests that this *spinster* could be most beneficial to surviving starvation out of the four other genes examined.
3. Further studies are required to determine the exact mechanism by which Spinster is involved in lysosome function and interacts with the autophagy pathway. Ultimately, it may contribute to development of new drug therapies for human lysosmal storage diseases such as neural ceroid lipofuscinoses and Tay Sachs disease.

References

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3. C. Settembre *et al.*. *Human molecular genetics* 17, 119 (Jan 1, 2008).

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