Deconvoluting the TGFβ aging pathway in *Caenorhabditis elegans*

As the story of the search for the 'fountain of youth' exemplifies, a way to extend human lifespan has been an obsession for centuries. Researchers using the microscopic nematode *Caenorhabditis elegans* have identified many simple genetic mutations that drastically alter lifespan. For example, a single-nucleotide change in the insulin receptor *daf*-2 doubles *C. elegans* lifespan, and a similar mutation also extends lifespan in mice and humans (Kenyon 1993, Bluher 2003, Kenyon 2010). *C. elegans*, due to its rapid life cycle and genetic simplicity, is an established model for studying the well-conserved genetic mechanisms of aging.

The TGFβ signaling pathways, which regulate growth and development, may also play a role in determining lifespan. Recently, studies in mice suggest that TGFβ may have age-dependent effects in mammals (Egerman 2015). In *C. elegans,* there are two TGFβ pathways that are thought to work independently to control body size and the development of stress-resistant dauer larvae. Mutations in the TGFβ ligands or type I receptors of either pathway cause minute changes in lifespan (Fletcher 2017, Mansfeld 2015). Remarkably, a mutation in the type II receptor, shared between both TGFβ pathways, causes a dramatic lifespan extension (Shaw 2007, Mansfeld 2015). This emergent trait suggests a redundantly shared role between the two TGFβ pathways. If this is the case, then mutations that span both TGFβ pathways will cause lifespan extension, while mutations that are localized to only one of the two pathways will not change lifespan. This proposal aims to (1) determine whether the two *C. elegans* TGFβ pathways redundantly control lifespan, and (2) investigate the downstream genetic mechanisms through which TGFβ controls lifespan.

The objective of this proposal is to investigate how two TGFβ pathways act together to regulate lifespan in *C. elegans*. To that end, I propose the following specific aims:

Aim 1: Determine whether the two *C. elegans* TGFβ pathways act redundantly to control lifespan. I will use a genetic approach to test the hypothesis that lifespan extension only occurs when both TGFβ pathways are off.

Aim 2: Identify genes that act downstream of both TGFβ pathways to control lifespan. I will use a functional-genomic approach to test the hypothesis that genes regulated redundantly by the two TGFβ pathways control lifespan.

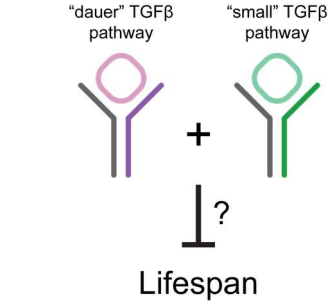

Figure 1: Do two TGFβ pathways act together to regulate lifespan? The TGFβ ligands are shown as rounded squares. The shared type II receptor is shown in grey.

	"Dauer" TGFβ	"Small" TGFβ
Ligand	*daf-7*	*dbl-1*
Type I Receptor	*daf-1*	*sma-6*
Type II Receptor	*daf-4*	*daf-4*

Table 1: Genes coding for the components of the two TGFβ pathways. The "dauer" TGFβ ligand *daf-7* signals through a receptor complex composed of *daf-1* and *daf-4* to control the development of the dauer larvae. The "small" TGFβ ligand *dbl-1* signals through a receptor complex composed of *sma-6* and *daf-4* to control body size (Gumienny 2013).

Experimental approach

Aim 1 is to determine whether the two *C. elegans* TGFβ pathways act redundantly to control lifespan (Figure 1). I will test the survival of *C. elegans* strains with null mutations in the type I receptors *daf-1* and *sma-6* alone, and *daf-1* and *sma-6* together (Table 1). My expectation is that the strain with both *daf-1* and *sma-6* mutations will live longer than either single-mutant. I will follow-up by testing the ligands *daf-7* and *dbl-1* alone and in combination with each other and the type II receptor *daf-4*. If the hypothesis that the double mutants will live longest is refuted, this will lead to the conclusion that *daf-4* regulates lifespan through another pathway.

Aim 2 is to identify genes that act downstream of the TGFβ pathways to control lifespan. I will test gene expression in *daf-1*, *sma-6*, and *sma-6; daf-1* mutants via microarray chips to determine genes differentially expressed between the single and double mutants. If the hypothesis in *Aim 1* is refuted, I will test gene expression of *daf-4*. I will follow-up by obtaining null mutations of or by inhibiting differentially expressed genes and recording lifespan.

Methods

The mutants to be tested in **Aim 1** will be provided by the Apfeld lab or combined using standard genetic techniques. Additional strains, if needed, will be provided by the Caenorhabditis Genetics Center (CGC). Strains to be tested will be grown under normal conditions until late adolescence, when they will be transferred to plates containing agar supplemented with fluorodeoxyuridine to prevent reproduction. I will record lifespan using a tool called the 'Lifespan Machine' which is designed to obtain large-scale lifespan data (Stoustrup 2013, Figure 2). The machine will take images every 15-60 minutes. I will use the Lifespan Machine software to infer a survival curve based on the images. I will subsequently annotate the curves manually to correct for any machine error, re-generate them, and analyze via proportional hazard and other established statistical models.

I will complete **Aim 2** using a similar approach to that taken previously to determine other lifespan-influencing genes (Murphy 2003). RNA will be extracted from wild type and mutant worms, reverse transcribed to cDNA, and run on Affymetrix microarray chips provided by the Boston University Microarray and Sequencing Resource facility. Differentially expressed genes will be identified through R, Bioconductor packages such as limma, and JMP (Richie 2015). I will then record lifespan of strains with mutations in identified genes. Mutant strains will be obtained from the CGC or, if such a strain is not available, I will inhibit the target gene via RNAi.

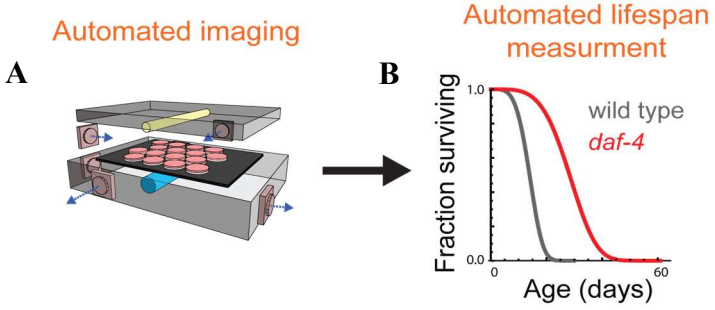

Figure 2: I will use an automated imaging platform to obtain high-resolution survival curves and evaluate differences in lifespan between mutant strains. (A) The Lifespan Machine scanner, which images around six-hundred individuals at once. (B) Sample data showing that *daf-4* mutants live significantly longer than wild-type.

Timeline

Aim 1 will take place in the first six months of this project, and **Aim 2** will be completed in the remaining eighteen months. These deadlines are reasonable in part due to my previous work with the Apfeld lab. Since October 2015, I have used genetic and statistical methods to investigate the interactions between transcription factors and TGFβ-pathway genes in oxidant resistance and lifespan. In the process, I have optimized protocols and created mutant strains that will aid in the completion of this project.

Summary and Significance

When completed, this project will elucidate both the interactions between the two *C. elegans* TGFβ pathways and the downstream mechanisms through which they regulate lifespan. I will combine my experience in wet-lab molecular biology with my interest in computational methods and functional genomics to collect rigorous data and make well-supported conclusions about the regulation of lifespan. In doing so, I will contribute to our understanding of the mechanisms that control lifespan in *C. elegans* and lay the foundation for similar studies in other organisms.

References

Bluher M., et al. (2003) Extended Longevity in Mice Lacking the Insulin Receptor in Adipose Tissue. Science 299(5606):572-574.

Egerman, M., et al. (2015) GDF11 increases with age and inhibits skeletal muscle regeneration. Cell Metabolism 22(1):164-174.

Fletche, M. and Kim, D. (2017) Age-dependent neuroendocrine signaling from sensory neurons modulates the effect of dietary restriction on longevity of *Caenorhabditis elegans.* PLOS Genetics 1006544.

Gumienny T.L., Savage-Dunn C. (2013) TGF-β signaling in C. elegans. WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.22.2.

Kenyon, C. (2010) The genetics of ageing. Nature 464: 504-512.

Mansfeld, J., et al. (2015) Branched-chain amino acid catabolism is a conserved regulator of physiological ageing. Nature Communications 5:10043.

Murphy, C. (2003) Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. Nature 424:277-84.

Ritchie, M., et al. (2015) *limma* powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research 43(7):e47.

Shaw, W., et al. (2007) The C. elegans TGF-beta Dauer pathway regulates longevity via insulin signaling. Curr Biol. 17(19):1635-45.

Stroustrup, N., et al. (2013) The *C. elegans* lifespan machine. Nature Methods 10:665-670.