RNA binding proteins coordinately control lifespan in *C. elegans*

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Introduction

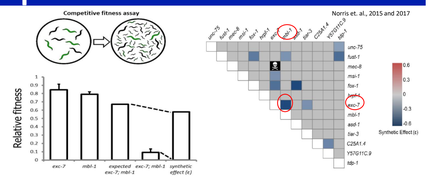
- While transcription factors have been extensively studied for their role in aging, less is known about how RNA binding proteins may contribute to the aging process
- We recently performed a CRISPR-SGI screen in *C. elegans* focused on conserved neuronally-expressed RNA binding proteins, and identified many double mutants with fitness defects.
- In one notable interaction between *mbl-1* and *exc-7*, double mutants displayed a severely shortened lifespan (~70%)
- We have used RNA seq data to investigate which RNAs may be uniquely dysregulated in the *mbl-1;exc-7* double mutant
- Our preliminary data has led us to hypothesize that immune gene dysregulation may play an important role in the shortened lifespan phenotype
- We are currently conducting tissue specific rescue as well as investigating genes of interest identified in our RNAseq analysis

Specific RNA binding proteins double mutants result in lifespan defects

- CRISPR/Cas-9 based Synthetic

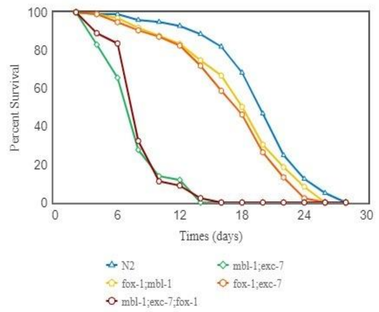
Genetic Interaction (CRISPR-SGI) screen

Identified fitness defects in mutants



Fitness deficit found in CRISPR-SGI Screen. Is it lifespan related?

- Aging assays (lifespan)



Observations from repeated experiments:

- mbl-1;exc-7 ~60-70% decrease in lifespan
- fox-1;exc-7 ~15-20% decrease in lifespan
- fox-1;mbl-1 ~ 10-15% decrease in lifespan
- mbl-1;exc-7;fox-1 ~58-70% decrease in lifespan

Figure 1. Representative aging assay for wildtype (N2), mbl-1;exc-7, fox-1;exc-7, mbl-1;fox-1, as well a mbl-1;exc-7;fox-1 mutants. Percent survival of 100 worms/genotype is shown over time. Restricted means: N2 (20.18 days), mbl-1;exc-7 (8.04 days), fox-1;mbl-1 (18.16 days), fox-1;exc-7 (17.45 days), mbl-1;exc-7;fox-1 (8.54 days)
(Han et. al., 2016)

We have identified a trio of RNA binding proteins combinatorially required for proper lifespan in *C. elegans*.

Healthspan assays revealed mbl-1;exc-7 mutants are healthy young worms with declining health with age

- Pumping assays (healthspan)

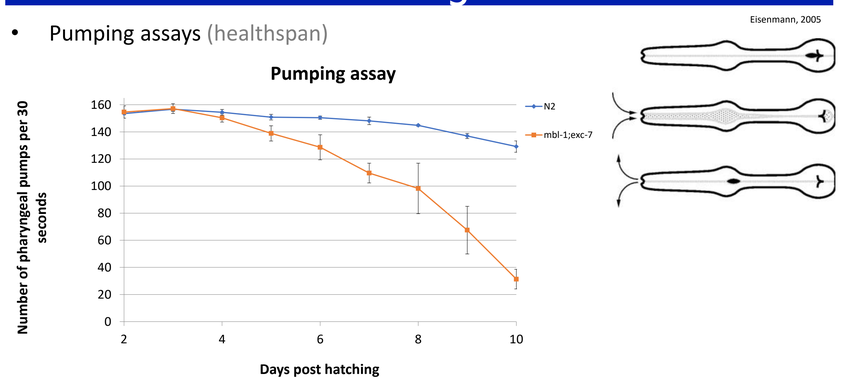


Figure 3. Data from four separate pumping assays. The number of pharyngeal pumps was recorded for 5 worms of each genotype from 2 -10 days post hatching

Pumping rates of the mbl-1;exc-7 mutants is similar to wildtype from L4-2 days into adulthood after which it starts declining more rapidly than in wildtype worms.

Healthspan assays reveal mbl-1;exc-7 mutants are healthy young worms with declining health with age

When does the mbl-1;exc-7 mutants' health start to decline?
Do they develop into healthy adults or do they have defects from during development?

- Thrashing assays (healthspan)

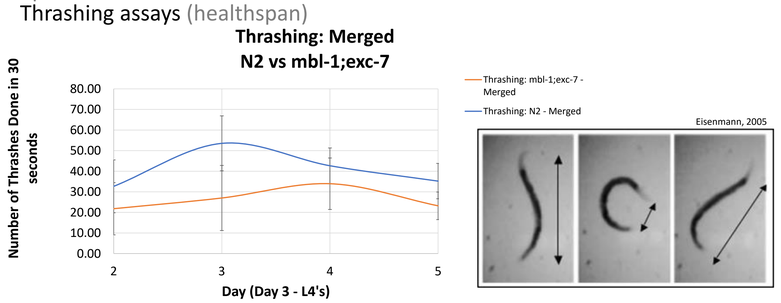


Figure 2. Data from three separate thrashing assays. The number of thrashes per 30 seconds was recorded for 5 worms per genotype from one day post hatching- early adulthood.

No significant thrashing defect is noted in the double mutant during early developmet-2 days into adulthood.

mbl-1;exc-7 mutants lay fewer eggs

- Egg laying assays (fertility)

Number of eggs/worm/day

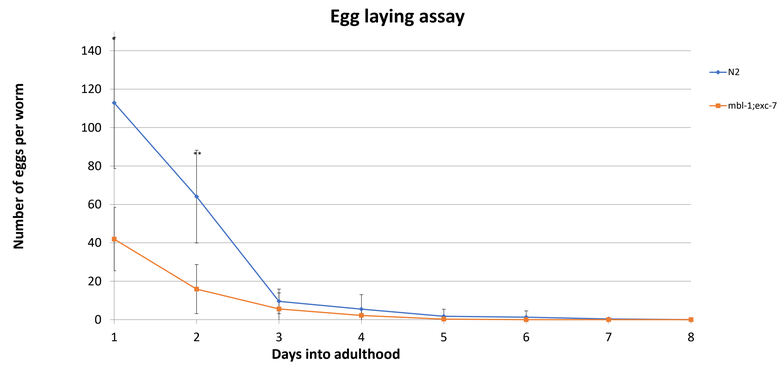


Figure 4. Data from four separate egg laying assays. The number of eggs laid/per worm was calculated for 5 worms per genotype from 1 day of adulthood until the end of egg laying. * p-value = 9.97E-05, ** p-value = 0.0008.

Mbl-1;exc-7 mutants lay significantly fewer eggs than wildtype worms

RNAseq analysis reveals many gene expression changes unique to mbl-1;exc-7 double mutants

- RNAseq analysis

Identified gene dysregulation, cassette exon events, retained introns, alternative 3' splice site, and alternative 5' splice site use unique to double mutant

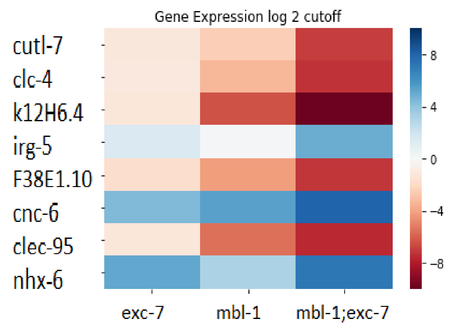
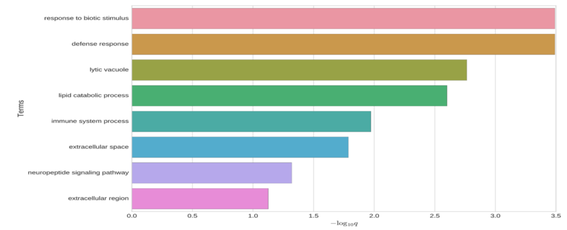


Figure 5. Heat map of significantly dysregulated gene expression of single exc-7, mbl-1, and double mutant mbl-1;exc-7 compared to wildtype (N2) worms at the L4 stage. Blue= upregulation, red= downregulation vs N2. Log 2 fold cutoff, P-value 0.05 cutoff.

We used a 0.05 significance and log2 fold cutoff to filter our RNAseq data in order to focus on the most highly dysregulated genes in the mbl-1:exc-7 double mutants.

Is the immune system involved in the lifespan phenotype?



Term	Expected	Observed	Enrichment Fold Change	P value	Q value
response to biotic stimulus	0.22	4	19	2.6e-06	0.00032
defense response	0.22	4	19	2.6e-06	0.00032
lytic vacuole	0.066	2	30	4.2e-05	0.0017
lipid catabolic process	0.082	2	24	8.1e-05	0.0025
immune system process	0.15	2	14	0.00043	0.011
extracellular space	0.18	2	11	0.00079	0.016
neuropeptide signaling pathway	0.076	1	13	0.0027	0.048
extracellular region	0.34	2	5.9	0.0048	0.075

Figure 6. Gene ontology information for mbl-1;exc-7 double mutant statistically significant gene dysregulation. (Angeles-Albores et. al., 2018)

Our RNA seq analysis and gene ontology on the significantly dysregulated genes showed immune system involvement. Leading us to investigate intestinal permeability and look into immune genes (e.g. irg-5)

- SMURF assays

(intestinal permeability/immune involvement)

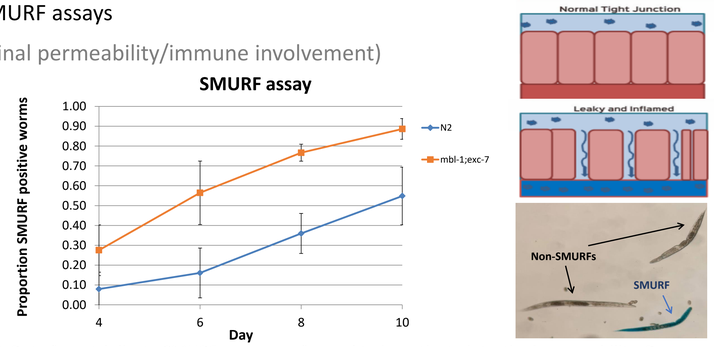


Figure 7. Data from triplicate SMURF assays performed to investigate intestinal permeability. Days= days post hatching. 20+ worms were used per genotype at each timepoint.

Mbl-1;exc-7 mutants show susceptibility to early intestinal permeability and suggest immune system dysregulation/vulnerability

mbl-1;exc-7;nhx-6 triple mutant partially rescues lifespan phenotype

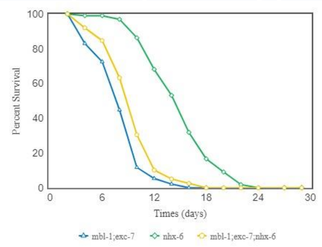


Figure 8. Representative aging assay for wildtype (N2), mbl-1;exc-7, nhx-6, and mbl-1;exc-7;nhx-6 mutants. Percent survival of 100 worms/genotype is shown over time. Restricted means: days), mbl-1;exc-7 (8.39 days), nhx-6 (15.24 days), mbl-1;exc-7;nhx-6 (9.76 days). mbl-1;exc-7 vs mbl-1;exc-7;nhx-6 corrected p-value = 0.0022
(Han et. al., 2016)

Nhx-6 deletion partially rescues the lifespan phenotype seen in mbl-1;exc-7 mutants

Future Directions

We are currently investigating further genes of interest (GOI) identified through our RNA seq analysis, and testing whether they modulate the lifespan phenotype of *mbl-1;exc-7* mutants. *mbl-1, fox-1,* and *exc-7* are neuronally-enriched genes and we are currently conducting experiments to test whether their expression in the nervous system is the critical tissue affecting whole-worm lifespan.

Citations and funding

Angeles-Albores, D; Lee, RYN; Chan, J; Sternberg, PW (2018): Two new functions in the WormBase Enrichment Suite. Micropublication: biology. Dataset. https://doi.org/10.17912/W25Q2N
Calarco, J. A., & Norris, A. D. (2018). Synthetic Genetic Interaction (CRISPR-SGI) Profiling in *Caenorhabditis elegans*. *Bio-protocol*, *8*(5), e2756. https://doi.org/10.21769/BioProtoc.2756
Eisenmann, D. M., Wnt signaling (June 25, 2005), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.7.1, http://www.wormbook.org.
Han S. Kyu, Lee D., Lee H., Kim D., Son H. G., Yang J., Lee S. V., Kim S. OASIS 2: online application for survival analysis 2 with features for the analysis of maximal lifespan and healthspan in aging research. *Oncotarget*. *2016*; *7*: 56147-56152. Retrieved from http://www.oncotarget.com/index.php?journal=oncotarget&page=article&op=view&path%5B%5D=11269

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