The effects of MsrA and MsrB in anoxia tolerance in aging Drosophila *melanogaster*

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Abstract

Drosophila *melanogaster* can withstand hours of oxygen deprivation (anoxia) by entering a protective coma called spreading depression. When oxygen is reintroduced to cells, a burst of reactive oxygen species (ROS) causes oxidative damage. Methionine is susceptible to oxidation to form methionine sulfoxide. This oxidation is reversible by the activity of methionine sulfoxide reductase (Msr) A and B, which reduce the S and R enantiomers, respectively. In this study, MsrA and MsrB single deletion mutants were exposed to one hour of anoxia and the Drosophila Activity Monitor (DAM) recorded their recovery times. RNA interference (RNAi) lines were used to mimic the effect of these deletion lines by ubiquitously knocking down their expression. **My current data indicates a significant difference in recovery time for the Msr mutants in middle age, but not near senescence.** Insight into the role(s) of Msr genes under anoxic stress could lead to a better understanding of how these genes contribute to aging.

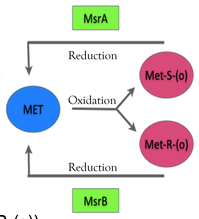
Introduction

Drosophila melanogaster

- Enters a protective coma that allows it to withstand hours of anoxia.

Reactive Oxygen Species (ROS)

- Oxygen reintroduced back into system
- Causes cellular damage: amino acid oxidation
- Free Radical Theory of Aging: Oxidation major contributor to aging



Methionine Sulfoxide Reductase (Msr)

- Methionine: most sensitive amino acid
- Methionine sulfoxide (A & B enantiomers)=Methionine oxidized
- MsrA's enzyme reduces the oxidized S enantiomer (met-S-(o))
- MsrB's enzyme reduces reduces the oxidized R enantiomer (met-R-(o))

Preliminary Data

- MsrA or MsrB single mutations or mutations in both genes result in longer recovery from protective coma.
- Environmental stress causes proteins to be induced to above normal levels in response to stress. The freshwater turtle, *Trachemys scripta*, does up-regulate expression of *MsrA* and *MsrB* in response to **anoxia**.

Fly Lines

Objective

I am proposing to extend these studies by examining whether the Msr genes are up-regulated during anoxia in Drosophila. RNAi lines will also be used to mimic the single loss of function (LOF) mutations by knocking down expression of either *MsrA* or *MsrB*, ubiquitously in the fly.

Msr Single LOF Lines

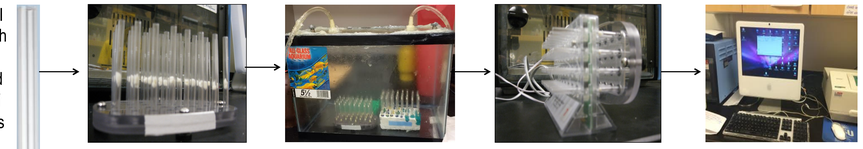
Genotype	MsrA	MsrB
WT60	Present	Present
A90	Not Present	Present
B54	Present	Not Present

RNAi Lines

Line	Genotype	MsrA	MsrB
RNAi-MsrA Control	w; UAS-RNAi-MsrA/ +;+	Present	Present
RNAi-MsrB Control	w; UAS_RNAi-MsrB/ +;+	Present	Present
Act-Gal4 Control	w; Act5c-Gal4/ +;+	Present	Present
Ubiquitous MsrA Knockdown	w; Act5c-Gal4/ UAS-RNAi-MsrA;+	Not Present	Present
Ubiquitous MsrB Knockdown	w; Act5c-Gal4/ UAS-RNAi-MsrB;+	Present	Not Present

Materials and Methods

1) Individual flies for each genotype were placed into each of the 32 tubes in the DAM holder.



2) The flies were in the DAM holder for a stabilization period of 10 minutes.

3) The flies were placed into the anoxia tank for 1 hour.

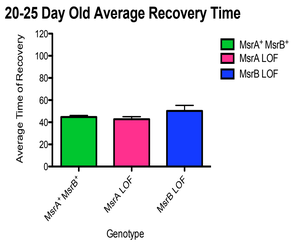
4) The flies were taken out of the anoxia tank and placed into the DAM system for 5 hours.

5) Analyzed and averaged the recovery times recorded by the DAM system

Results

1. The WT60 line recover significantly later as they approach senescence, while the MsrA and MsrB mutants reach maximum recovery time at middle age (40-45 days old).

A.



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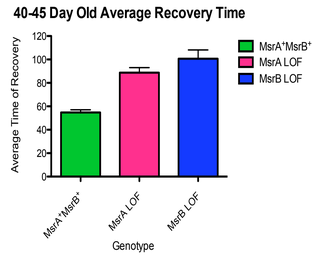

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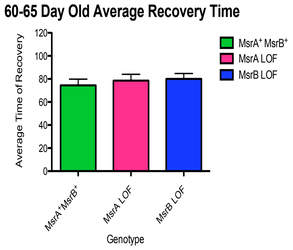


D.

MsrA⁺MsrB⁺ vs. MsrA LOF	MsrA⁺MsrB⁺ vs. MsrB LOF	MsrA LOF vs. MsrB LOF
p=0.4720	p=0.2936	p=0.1791

E.

MsrA⁺MsrB⁺ vs. MsrA LOF	MsrA⁺MsrB⁺ vs. MsrB LOF	MsrA LOF vs. MsrB LOF
p<0.0001	**p<0.0001**	**p=0.1713**

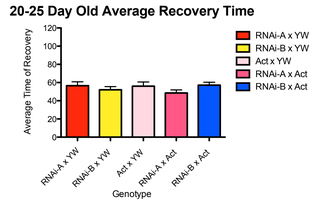
F.

MsrA⁺MsrB⁺ vs. MsrA LOF	MsrA⁺MsrB⁺ vs. MsrB LOF	MsrA LOF vs. MsrB LOF
p=0.1124	p=0.5190	p=0.8899

Figure 1: There is no significant difference in average recovery time at young age (20-25 days) between MsrA⁺MsrB⁺ and either single Msr mutant (1D). The average recovery time for MsrA⁺MsrB⁺ (WT60) increases as the fly ages.There is a significant difference in average recovery time for each 40-45 day old Msr mutant compared to WT60 (1E). The MsrB mutant takes significantly longer than the MsrA mutant to recover from anoxic stress (p=0.0186). At 60-65 days, there is no significant difference in recovery time between the Msr mutants (p=0.2203), indicating that the mutants reached maximum recovery time at middle-age (40-45 days).

2. The RNAi-A and RNAi-B knockdown lines reach maximum average recovery time after anoxic stress at middle age (40-45 days old).

A.



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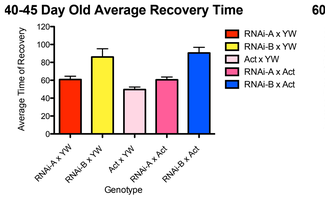

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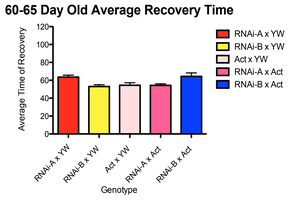

Figure 2: See Figure 3 analysis for the 20-25 days old, 40-45 days old, and 60-65 days old age groups.

Results

3. The RNAi-A and RNAi-B knockdown lines do not significantly differ in average recovery time during senescence.

A.

20-25 Day Old p values

Gene	RNAi x YW vs. Act x YW	RNAi x Act vs. Act x YW	RNAi x Act vs. RNAi x YW
MsrA	0.6296	0.3603	0.0658
MsrB	0.8490	0.8714	0.2374

B.

40-45 Day Old p values

Gene	RNAi x YW vs. Act x YW	RNAi x Act vs. Act x YW	RNAi x Act vs. RNAi x YW
MsrA	0.0069	0.0268	0.8755
MsrB	<0.0001	<0.0001	0.5557

C.

60-65 Day Old p values

Gene	RNAi x YW vs. Act x YW	RNAi x Act vs. Act x YW	RNAi x Act vs. RNAi x YW
MsrA	0.0958	0.06449	0.0023
MsrB	0.7292	0.0510	0.0213

Figure 3: As the flies approach senescence, a significant difference in average recovery time is not seen in the 60-65 day lines as indicated by Figure 3C.

Discussion

- The results obtained slightly deviated from my original hypothesis that stated the MsrA and MsrB deletion lines as well as the RNAi-lines will take significantly longer to recover as the fly approaches senescence.
- The MsrA⁺MsrB⁺ (WT60) line recovers significantly later as the flies approached senescence in comparison to the MsrA and MsrB deletion lines. This fast average recovery time is expected among the WT60 line because this line contains both the MsrA and MsrB enzymes that play a protective role in reducing the oxidized forms of methionine.
- A significant difference is not seen in average recovery time between the 20-25 days old MsrA deletion line and the MsrB deletion line. Their RNAi lines also show no significant difference in average recovery time between the parental lines and their progeny.
- The RNAi lines knock down the expression of the MsrA or MsrB gene to mimic the deletion effect in the deletion lines. The MsrA and MsrB deletion lines at 60-65 days recover faster than the 40-45 day old deletion lines. Similar results are seen in their respective RNAi lines, although there are a few discrepancies because the RNAi-lines may contain residual forms of the MsrA or MsrB gene.
- As the flies grew older, the decrease in average recovery time among the MsrA and MsrB deletion lines, indicate that the MsrB gene is crucial in counteracting anoxic stress conditions, especially as the flies are aged.

Future Work

Repeating single Msr deletion and RNAi experiments with young (20-25), middle (40-45), and old (60-65) day old flies

Performing a Western Blot on the Msr single deletion lines under **1) normoxia, 2) anoxia, 3) reoxygenation conditions**

Extend RNAi experiments using tissue specific drivers: **OK6** (Motor Neurons) & **GAWB** (Muscle)

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Reference: Milton et al., 2005,S.L. Milton, H.M. Prentice, P.L. Lutz, Molecular mechanisms of ROS defense in the turtle Trachemys scripta Comp. Biochem. Physiol., 141A (2005), p. S185