Expression of Thor does not increase desiccation resistance in Drosophila melanogaster

Robert L. Kobey
*University of Nevada Las Vegas*

Deborah K. Hoshizaki
*University of Nevada Las Vegas*

Allen G. Gibbs
*University of Nevada Las Vegas, School of Life Sciences, Mentor*

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Desiccation Resistance and THOR

Using microarray analysis of Drosophila melanogaster, the Gibbs lab has identified several hundred candidate genes that may be involved in desiccation resistance. One of these genes is Thor, an important downstream target of the TOR/insulin signaling pathway. Preliminary results confirm that Thor plays a role in desiccation resistance. Further research will be needed to verify these results and understand the mechanism by which Thor increases desiccation resistance. This research will also serve as a proof-of-principle for testing microarray-derived hypotheses.

A previous microarray analysis found evidence that down-regulation of protein synthesis might be a cellular response to desiccation through the up-regulation of Thor. When Drosophila melanogaster adult males are exposed to desiccation, Thor expression increases 6.5-fold. Thor codes for the D. melanogaster 4E-binding protein (4E-BP), which inhibits translation by binding to the eukaryotic initiation factor 4E (eIF-4E). Thus, a reduction in protein synthesis might function to reduce energy expenditures during desiccation. To test whether THOR plays a role in the response to desiccation, we measured desiccation resistance in flies with altered Thor expression. We measured desiccation resistance in flies with Thor expression reduced through P-element mutagenesis (Thork13517 and Thor2) and RNA interference (RNAi). Using the GAL4/UAS system (Brand and Perrimon, 1993), desiccation resistance was also measured in flies with increased expression of wild-type Thor and constitutively-active Thor (4E-BP(AA)). We found that Thor hypomorph mutant males (Thork13517) are desiccation sensitive. However, we found no difference in desiccation sensitivity between Thor null mutants (Thor2) and control flies (Thor1rv1). Knocking down expression of Thor with RNAi increased desiccation sensitivity. However, desiccation resistance did not increase in male flies that over-expressed Thor or a constitutively-active Thor (4E-BP(AA)) using the GAL4/UAS system. These mixed results do not support the hypothesis that Thor expression increases desiccation resistance.
Expression of Thor Does Not Increase Desiccation Resistance in *Drosophila melanogaster*

School of Life Sciences, University of Nevada, Las Vegas

Robert L. Kobey, Deborah K. Hoshizaki, and Allen G. Gibbs

Introduction

A previous microarray analysis found evidence that down-regulation of protein synthesis might be a cellular response to desiccation through the up-regulation of Thor. When *Drosophila melanogaster* adult males are exposed to desiccation, Thor expression increases 6.5-fold. Thor is a member of the D. melanogaster 4E-binding protein (4E-BP), which inhibits translation by binding to the eukaryotic initiation factor 4E (eIF-4E). Thus, a reduction in protein synthesis might function to reduce energy expenditures during desiccation.

To test whether THOR plays a role in the response to desiccation, we measured desiccation resistance in flies with altered Thor expression. We measured desiccation resistance in flies with Thor expression reduced through P-element mutagenesis (Thor<sup>W</sup> and Thor<sup>Wd</sup>) and RNA interference (RNAi). Using the GAL4/UAS system (Brand and Perrimon, 1993), desiccation resistance was also measured in flies with increased expression of wild-type Thor and constitutively-active Thor (Thor<sup>AE-4E-BP(4A)</sup>). We found that Thor hypermorph mutant males (Thor<sup>Wd</sup>) are desiccation sensitive. However, we found no difference in desiccation sensitivity between Thor-null mutants (Thor<sup>Wd</sup>) and control flies (Thor<sup>W</sup>). Knocking-down expression of Thor with RNAi increased desiccation sensitivity. However, desiccation resistance did not increase in male flies over-expressed Thor or a constitutively-active Thor (Thor<sup>AE-4E-BP(4A)</sup>) using the GAL4/UAS system. These mixed results do not support the hypothesis that Thor expression increases desiccation resistance.

Figure 1. Insulin/TOR Signaling Pathway. In response to reduced levels of ATP, AMP kinase is activated, resulting in the activation of TSC1/2 and subsequent inhibition of TOR. In the absence of active TOR, THOR can bind to eIF-4E and inhibit cap-mediated translation. (Adapted from Hoshizaki & Gibbs, 2007)

Methods

Fly Stocks

The Thor null mutant (Thor<sup>Wd</sup>), Thor hypomorph mutant (Thor<sup>W</sup>), genetic background control (Thor<sup>W</sup>), and UAS-Thor lines were provided by Deborah Kimbell (UC, San Francisco). The constitutively-active Thor line (UAS-4E-BP(4A)) was provided by Stephen Cohen (Boston University). The GAL4 driver line (Act<sup>UAS-gal4</sup>) was obtained from the Bloomington Stock Center.

Determination

Flies were reared at 25°C. Newly-emerged adult males were collected and aged for five days before desiccation. Groups of 50 flies were used for each genotype. Viability was recorded every hour and the number of survivors determined.

Water Content

Newly-emerged flies were collected and exposed for the desiccation assay between 8:00 am and 4:00 pm. The relative humidity was maintained at 25°C. 60 flies were weighed on a Cahn C-2 microbalance and 60 flies were dried overnight at 35°C and the dry mass determined. Water content was calculated as the difference between the two measurements.

Metabolic Rate and Water Loss Rate

We used a Y-2 respirometry system (Sable Systems, Las Vegas NV) to measure CO<sub>2</sub> production by groups of 50-60 flies. Flies were placed in 2-nL respirometry chambers. Dry CO<sub>2</sub>-free air was pumped at a flow rate of 50mL/min through the chambers to a 1.5L CO<sub>2</sub>-free infrared gas analyzer.

Results

Figure 2. Thor hypomorphs (Thor<sup>Wd</sup>) are more desiccation-sensitive than wild-type (Thor<sup>W</sup>) or null mutant (Thor<sup>W</sup>) flies. There is no significant difference in desiccation resistance of the wild-type and null mutant flies. Thor hypomorphs have a higher metabolic rate and a higher water-loss rate than null mutants and wild-type.

Figure 3. Knocking-down expression of Thor with RNAi (Act<sup>UAS-gal4/UAS-Thor RNAi</sup>) increases desiccation sensitivity compared to parental (Act<sup>UAS-gal4/Cyo</sup> and UAS-Thor RNAi) and sibling (UAS-Thor RNAi/Cyo) controls. Thor knock-down flies (Act<sup>UAS-gal4/UAS-Thor RNAi</sup>) have a higher water-loss rate than controls.

Figure 4. Expressing constitutively-active Thor (Act<sup>UAS-gal4/Thor AE-4E-BP(4A)</sup>) does not increase desiccation resistance compared to parental (Act<sup>UAS-gal4/Cyo</sup> and UAS-Thor RNAi) and sibling (UAS-Thor RNAi/Cyo) controls. Flies expressing constitutively-active Thor have a higher water content, metabolic rate, and water-loss rate.

Figure 5. Overexpressing wild-type Thor (Act<sup>UAS-gal4/UAS-Thor</sup>) does not increase desiccation resistance compared to parental (Act<sup>UAS-gal4/Cyo</sup> and UAS-Thor) and sibling (UAS-Thor RNAi/Cyo) controls.

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References


Figure 3. Insulin Signaling Pathway. The insulin receptor (IR) and its binding partners (Akt, P38, PDK1) are shown. The Akt pathway is then shown, culminating in the activation of mTOR, a key regulator of protein synthesis and cell growth. (Adapted from Hoshizaki & Gibbs, 2007)