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Expression of Thor does not increase desiccation resistance in Drosophila melanogaster

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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*

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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* 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 (*Thor*[illegible] and *Thor*^{2}) 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 (*Thor*[illegible]) are desiccation sensitive. However, we found no difference in desiccation sensitivity between *Thor* null mutants (*Thor*^{2}) and control flies (*Thor*[illegible]). 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.

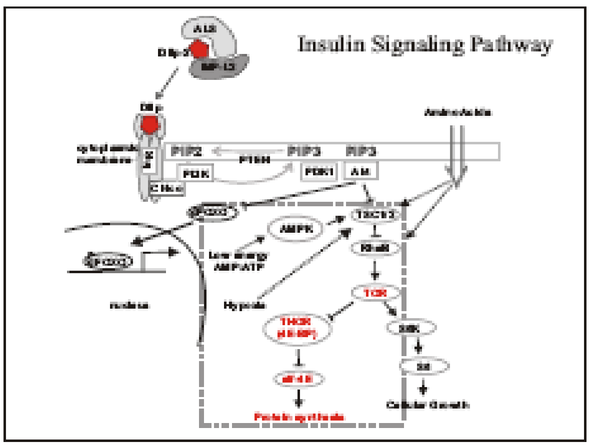

Figure 1. Insulin/TOR Signaling Pathway. In response to reduced levels of ATP, AMP kinase is activated, resulting in the activation of TSC1/2 and subsequent inhibition of TOR. In the absence of active TOR, THOR can bind to eIF-4E and inhibit cap-mediated translation. (Adapted from Hoshizaki & Gibbs,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

Methods

Fly Stocks

The *Thor* null mutant (*Thor*^{2}), *Thor* hypomorph mutant (*Thor*[illegible]), genetic background control (*Thor*[illegible]), and *UAS-Thor* lines were provided by Deborah Kimbrell (Univ. of California, Davis). The constitutively-active *Thor* line (*UAS-4E-BP(AA)*) was provided by Stephen Cohen (Temasek Life Sciences). The GAL4 driver line (*Actin5c-gal4*) was obtained from the Bloomington Stock Center.

Desiccation

Flies were reared at 25°C. Newly-eclosed adult males were collected and aged for five days before desiccation.

Groups of 5 flies/vial were desiccated at 25°C. A total of 50 flies were tested for each genotype. Vials were monitored every hour and the number of survivors determined.

Water content

Newly eclosed flies were collected as described for the desiccation assay and frozen at -80°C for < 24hr. Wet mass was measured to ± 0.001 mg using a Cahn C-29 microbalance. Adults were dried overnight at 55°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 TR-2 respirometry system (Sable Systems, Las Vegas NV) to measure CO_2 production by groups of ~10 male flies. Flies were placed in 5-mL respirometry chambers. Dry CO_2-free air was pumped at a flow rate of 50mL/min through the chambers to a LiCor LI-6262 infrared gas analyzer.

References

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118, 401-415.
Hoshizaki, D.K. and Gibbs, A.G. (2007). Meeting report: integrating insulin signaling and stress responses. Fly 1: 110-112.

Results

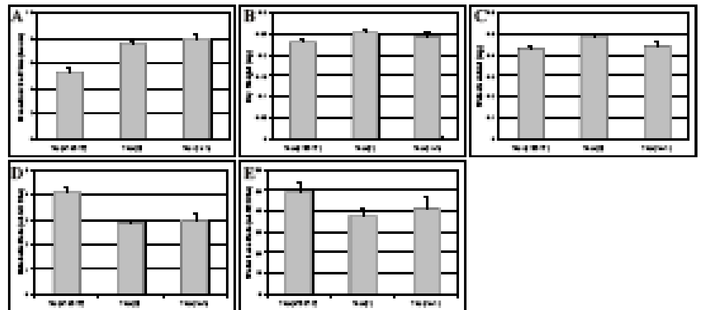

Figure 2. *Thor* hypomorphs (*Thor*[illegible]) are more desiccation sensitive than wild-type (*Thor*[illegible]) or null mutant (*Thor*^{2}) flies. There is no significant difference in the desiccation resistance of the wild-type and null mutant flies. *Thor* hypomorphs have a higher metabolic rate and a higher water-loss rate than null mutants and wild-type.

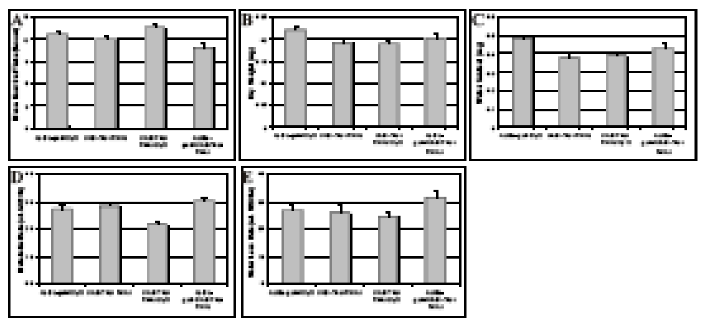

Figure 3. Knocking-down expression of *Thor* with RNAi (*Act5c-gal4/UAS-Thor RNAi*) increases desiccation sensitivity compared to parental (*Act5c-gal4/CyO* and *UAS-Thor RNAi*) and sibling (*UAS-Thor RNAi/CyO*) controls. *Thor* knock-down flies (Act5c-gal4/UAS-Thor RNAi) have a higher water-loss rate than controls.

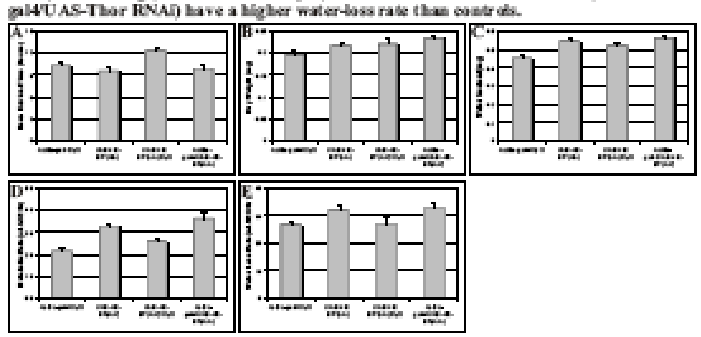

Figure 4. Expressing constitutively-active *Thor* (*Act5c-gal4/UAS-4E-BP(AA)*) does not increase desiccation resistance compared to parental (*Act5c-gal4/CyO* and *UAS-4E-BP(AA)*) and sibling (*UAS-4E-BP(AA)/CyO*) controls. Flies expressing constitutively-active *Thor* have a higher water content, metabolic rate, and water-loss rate.

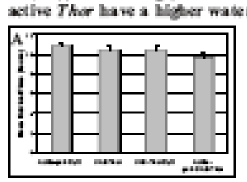

Figure 5. Overexpressing wild-type *Thor* (*Act5c-gal4/UAS-Thor*) does not increase desiccation resistance compared to parental (*Act5c-gal4/CyO* and *UAS-Thor*) and sibling (*UAS-Thor/CyO*) controls.

Acknowledgments

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