The Role of dTORC1 on Muscle Development, Function and Longevity in Drosophila

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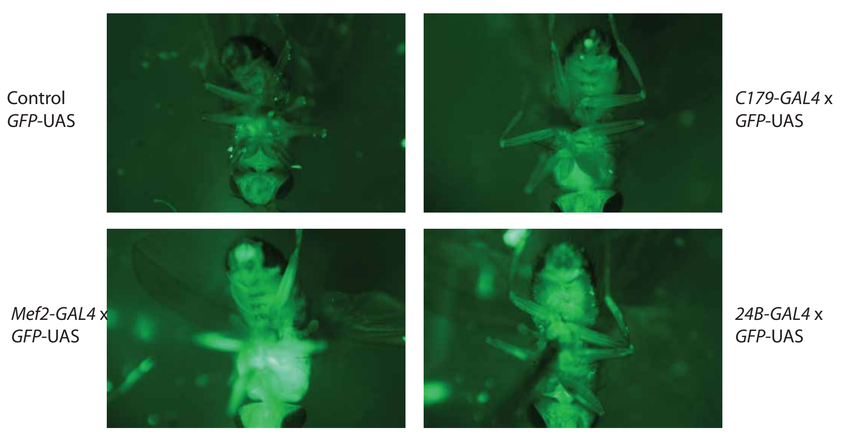
SUMMARY

The TORC1 signaling pathway is critical for cell growth and proliferation. It has been implicated in disorders ranging from diabetes and obesity to depression and cancer. Previous work has implicated the TORC1 pathway in the regulation of longevity and muscle function in a variety of model systems. In this study, we manipulated the activity of mTORC1 in muscle tissue by using the Drosophila GAL4/UAS system. We did this by knocking down both positive (*Raptor*) and negative (*Tsc1*) regulators of dTORC1 function in both cardiac and skeletal muscles. We observed that genetic inhibition of TORC1 in skeletal but not cardiac muscle leads to reduced viability using the skeletal muscle GAL4 drivers (*C179*-GAL4 and *24B*-GAL4). Using climbing assays, we have also examined the effects of these manipulations on muscle function and have observed reduced fly motility with both Raptor and Tsc1 inhibition in muscle. We found that activation of TORC1 in fly skeletal muscle tissue also leads to significant reductions in lifespan. Both the reduced muscle function and shortened lifespan are consistent with results obtained in a mouse model of muscle *Tsc1* deletion. Expression of both positive and negative regulators of TORC1 specifically in cardiac muscle using the *Hand*-GAL4 driver had no dramatic effects on either viability or longevity. These data provide insights into the role of muscle TORC1 activity in development, muscle function and longevity.

Genetic Manipulation of the TORC1 Complex

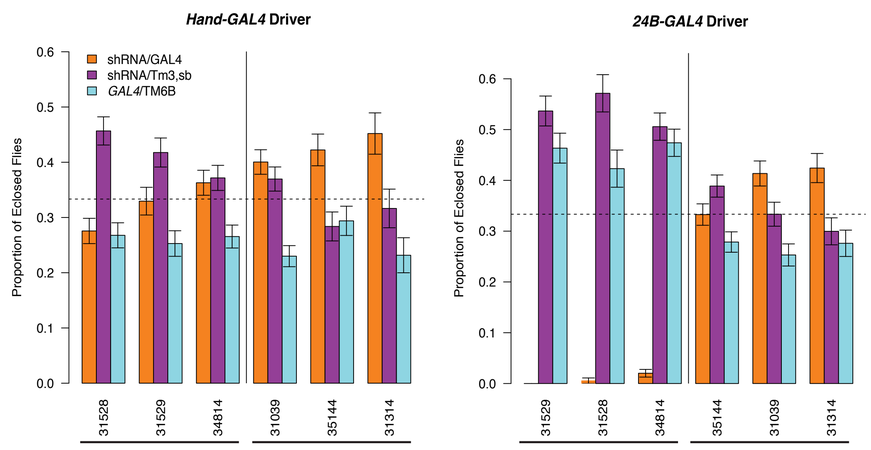
TORC1 is a nutrient sensing kinase affecting translation, transcription, autophagy, metabolism, growth, and cell survival. Inhibition of TORC1 has been shown to increase longevity in model organisms including flies. We studied the effects of muscle TORC1 on aging through the suppression of *Tsc1*and *Raptor*. When *Tsc1* is supressed, TORC1 is activated, and when Raptor is suppressed, TORC1 is downregulated. We used these tools, combined with muscle *GAL4* drivers to examine the role of this complex in muscle tissue.

Expression of Muscle *GAL4* Drivers

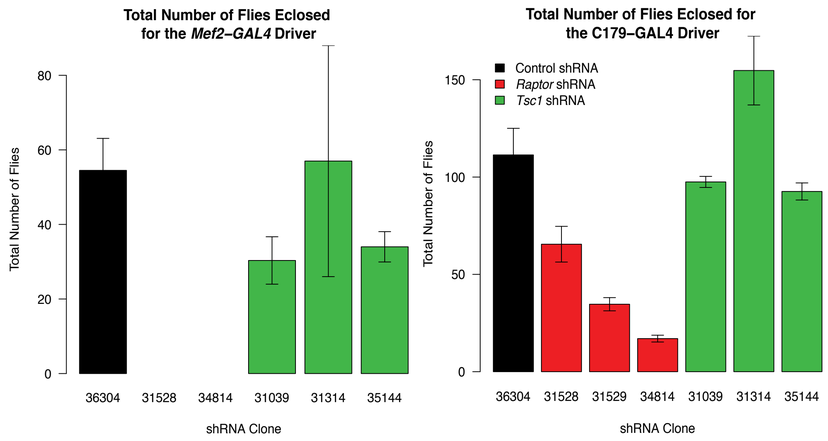


The *GAL4*/UAS system was used to drive the expression of *Tsc1* and *Raptor* shRNAs. Four different *GAL4* drivers were used to knock down *Tsc1* and *Raptor* suppression to specific tissues. The *Hand-GAL4* driver (not pictured) is active in cardiac muscle and the *C179-GAL4*, *24B-GAL4*, and *Mef2-GAL4* drivers are all active in skeletal muscle. These drivers were crossed with UAS-*GFP* and *GFP* was used to visualize expression of these *GAL4* Drivers.

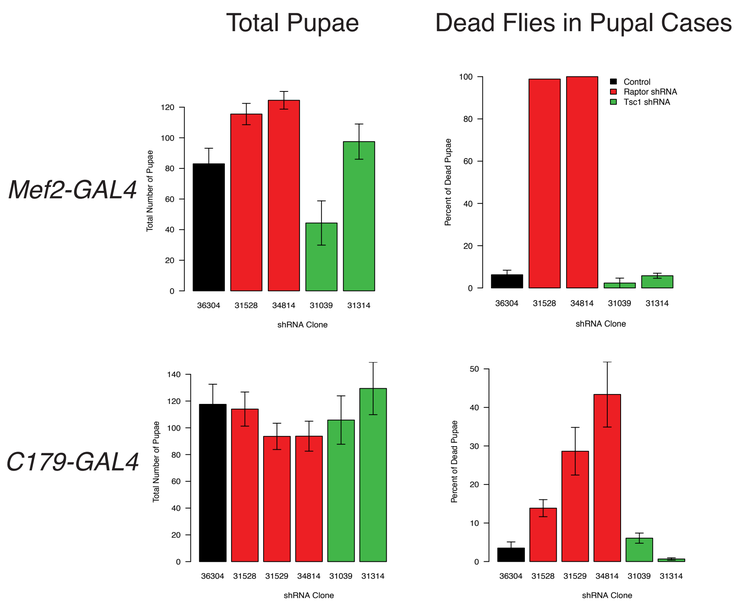
Knockdown of *Raptor* in Skeletal Muscle Causes Lethality



Heterozygous strains containing either the *GAL4* driver or the indicated shRNA over a balancer chromosome were crossed. Three different shRNAs for *Raptor* and *Tsc1* were used. The progeny containing both balancers were excluded from the analysis due to known reduced viability. Reducing *Tsc1* and *Raptor* in cardiac muscle using the *Hand-GAL4* driver produced no significant effects on the birth rates of the progeny. While there was no significant effect on knocking down *Tsc1* in skeletal muscle using the *24B-GAL4* driver, knocking down *Raptor* resulted in near complete lethality. The dashed line indicates expected mendelian ratios. Similar phenotypes were observed with *Mef2-GAL4* driven flies. *C179-GAL4* driven *Raptor* knockdown flies were partially lethal.

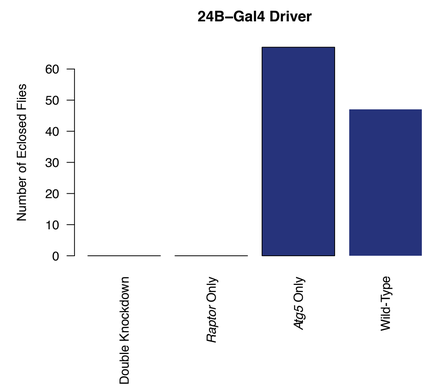


Raptor Knockdown in Muscle Causes Pupal Lethality



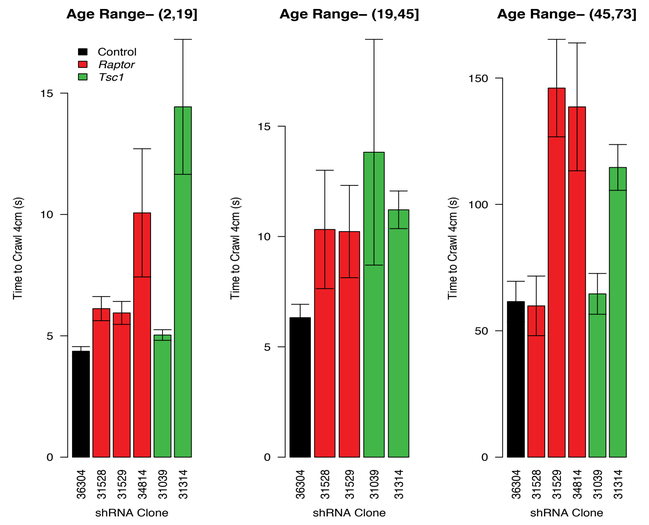
To determine when the *Raptor* knockdown flies were dying, the pupal cases were examined for dead flies. The dead pupae were indicated by a darkening and shrinking of the fly within the pupal case. In accordance with the lethality effect produced by the *Mef2-GAL4* driver, nearly all of the pupal cases of the *Raptor* knockdown progeny contained dead flies. There was also a high percentage of dead *Raptor* knockdown pupae from the crosses using the *C179-GAL4* driver. The number of dead pupae corresponds to the relative strengths of the *Raptor* shRNA, with the strongest resulting in the most dead pupae. These results suggest that the lethality effect associated *Raptor* suppression in skeletal muscle occurs at the pupal stage.

Knockdown of *Atg5* Does Not Rescue Lethality



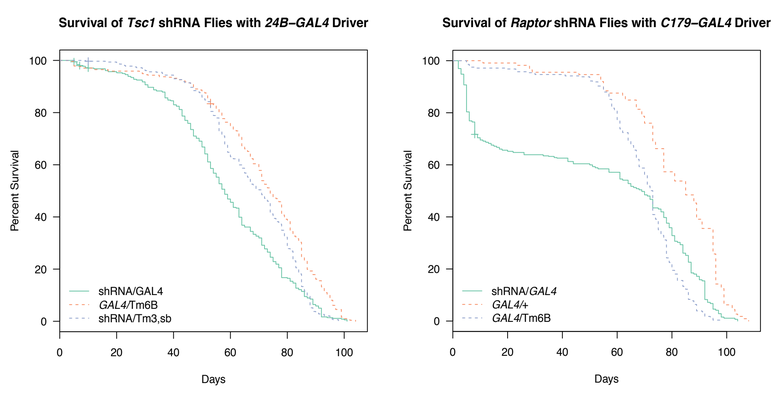
The suppression of *Raptor*, and therefore TORC1, results in increased levels of autophagy. To determine whether increased autophagy was the cause of *Raptor* knockdown lethality, crosses were created to produce double knockdown flies, expressing both *Raptor*-shRNA and *Atg5*-shRNA in skeletal muscle. Suppression of autophagy was unable to rescue the *Raptor* knockdown lethality effect, as no double knockdown flies were produced.

Raptor and *Tsc1* Knockdown Flies Have Impaired Climbing



To study the effect of TORC1 regulation on muscle function, the muscle strength of the progeny of the *C179-GAL4* crosses was measured approximately every 30 days. Muscle stength was determined by the amount of time it took each fly to climb 4 cm up the vial. *Tsc1* and *Raptor* knockdown flies generally took longer to climb up the vial than the controls. The results indicate that both *Tsc1* and *Raptor* suppression results in an accelerated decline in muscle strength.

Both *Raptor* and *Tsc1* Have Reduced Longevity



We observed reductions in lifespan for both muscle driven *Tsc1* and *Raptor* knockdown. For muscle driven *Tsc1* knockdown, flies died prematurely relative to their controls. In the case of *C179-GAL4* driven *Raptor* knockdown, the knockdown flies initially died off rapidly; however, the knockdown flies that lived past this critical period of about 20 days lived about as long as the controls. There was no dramatic effect of *Hand*-Gal4 (cardiac) driven knockdown of *Raptor* or *Tsc1*.