A Kinome RNAi Screen Reveals Genes Required for Muscle Tissue Maintenance

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Abstract

NUAK is an AMPK-related kinase which controls autophagy (cellular recycling) of an important linker protein in muscle called Filamin[1]. The **absence of NUAK prevents the turnover of Filamin, presents an accumulation of autophagic markers such as p62 and ATG8 and leads to muscle degeneration**. **The goal of our research was to determine which other kinase genes might be required to regulate autophagy** by turning them off with RNA interference (RNAi) and looking for abnormal muscle structure and attachment. 25 candidate kinase genes were selected and turned off in the muscle of *Drosophila melanogaster* larvae using RNAi. The larvae were then dissected and imaged under a laser confocal microscope to observe muscle structure. **Knockdown of seven kinase genes *yata, nippedA, CDK9, for, RIOK1, lic, and Vps15* showed abnormal muscle structure such as detachment and muscle thinning.** These kinase genes will be further analyzed for their requirement in the proper autophagy of Filamin.

Background

•**Why Use Flies?** **75% of known human disease genes have a similar gene in *Drosophila melanogaster***[2] and "…*Drosophila* NUAK shares 61% identity and 80% similar to human NUAK 1 and NUAK 2."[1] **Thus, fruit flies are excellent models for human muscle diseases** to uncover kinases similar to NUAK that may affect autophagy.

•**Therapeutic application:** Myofibrillar myopathies (MFM) are degenerative muscle diseases that share characteristics seen in *NUAK -/-* flies, **especially aggregation (accumulation/clumping) of Filamin.**[1] Discovering other kinases that are important for autophagy and preventing Filamin aggregation **has vital potential for developing therapies against MFM.**

HELPFUL DEFINITIONS:

•**Kinase:** a protein that **controls activation of cellular processes** by transferring a phosphate molecule (molecular "on/off switch")

•**RNAi:** a method used to **knockdown** the amount of a protein so it cannot function (used to "turn off" kinase)

•**Filamin: important linker protein in muscle**. Improper autophagy causes Filamin aggregation (clumping) and muscle degeneration

•**Autophagy: cellular recycling of old cell parts**. Recycling old Filamin is necessary for making new healthy Filamin

•**NUAK:** an AMPK-related kinase that controls the autophagy of Filamin

•**MHC-GFP:** a marker that **makes *Drosophila* muscle glow green** when illuminated under a laser confocal microscope

•**Mef2-Gal4:** genetic instructions that make sure the RNAi is produced in muscle. **Used to turn off kinase specifically in muscle**

•**UAS:** gene that interacts with Gal4 to **activate production of RNAi**

Methods

•Collected ~5 males from 25 different strains of kinase UAS-RNAi lines (each RNAi "turns off" a kinase in the larvae) and crossed to 10-15 MHC-GFP, Mef2-Gal4 virgin females in a vial with fly food and dry yeast (to help promote reproduction and healthy larvae).

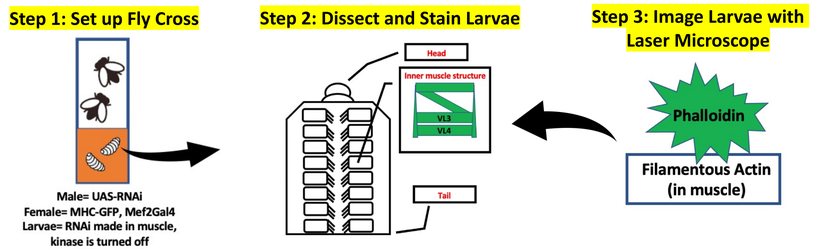
•Incubate at 29^{O} C until wandering L3 larvae are present (larvae crawl on side of tube)

•Collect 6-10 **wandering L3 larvae**, heat-kill with 65^{O} C water and dissect them

•Preserve the larvae with 4% formaldehyde, and **stain with Phalloidin** (binds to filamentous actin in muscle; glows under laser and **acts as fluorescent marker to be able to see muscle clearly**).

•Mount larvae on slides with fluorescent mounting media

•Imaged the VL3 and VL4 muscles of larvae with a laser confocal microscope, at 20x magnification and 0.5 zoom.



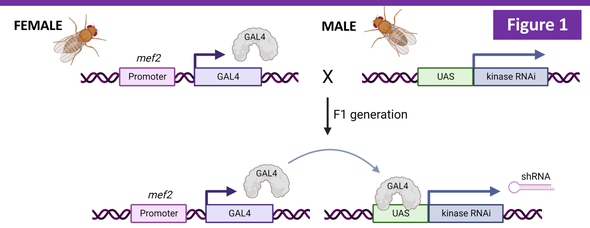


Fig. 1: Interaction of Gal4 and UAS to produce kinase RNAi

Results

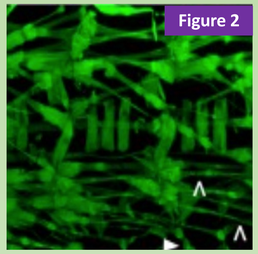


Fig. 2: NUAK -/- *D. melanogaster* L3 larvae[1]
Reference for abnormal muscle structure

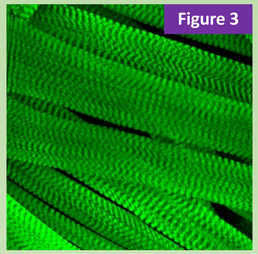


Fig. 3: lacZ x MHC-GFP, Mef2Gal4
D. melanogaster L3 larvae (CONTROL)

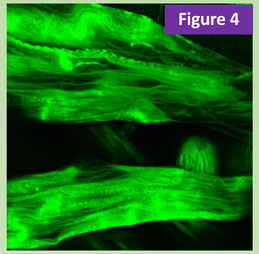


Fig. 4: 32986 x MHC-GFP, Mef2Gal4
D. melanogaster L3 larvae

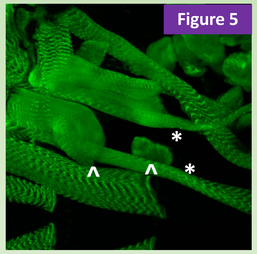


Fig. 5: 34849 x MHC-GFP, Mef2Gal4
D. melanogaster L3 larvae

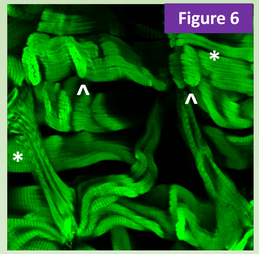


Fig. 4: 34982 x MHC-GFP, Mef2Gal4
D. melanogaster L3 larvae

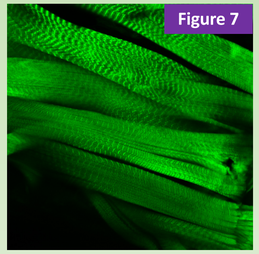


Fig. 7: 35158 x MHC-GFP, Mef2Gal4
D. melanogaster L3 larvae

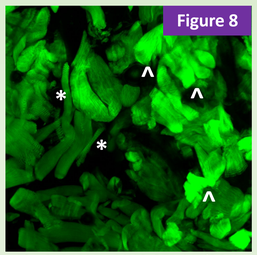


Fig. 8: 35293 x MHC-GFP, Mef2Gal4
D. melanogaster L3 larvae

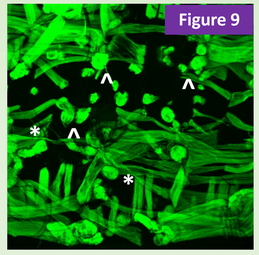


Fig. 9: 60010 x MHC-GFP, Mef2Gal4
D. melanogaster L3 larvae

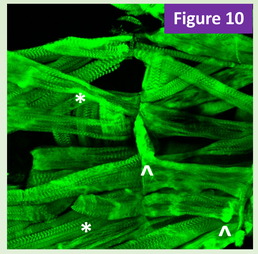


Fig. 10: 34092 x MHC-GFP, Mef2Gal4
D. melanogaster L3 larvae

Results

Table 1: Male *D. melanogaster* Fly Genotype and Corresponding Muscle Characteristics

Drosophila Stock Name	Corresponding RNAi Gene-Target	Characteristics in VL3 and VL4 Muscle Thinned (*), detached (^)
Lac Z	none	Normal muscle
32986	*yata*	Loss of sarcomeric patterning, gaps in muscle
34849	*nippedA*	Detached and thinned
34982	*CDK9*	Detached and thinned
35158	*for*	Loss of sarcomeric patterning, gaps in muscle
35293	*RIOK1*	Detached muscle
60010	*lic*	Detached and thinned
34092	*Vps15*	Loss of sarcomeric patterning, thinned and detached

Conclusions/Future Research

•From the screen, we identified **7 kinase genes** that when knocked down with RNAi showed characteristics **comparable to *NUAK-/-* flies.**

•These kinase genes were: ***yata, nippedA, CDK9, for, RIOK1, lic, Vps15.***

•Features observed upon kinase RNAi knockdown: muscle thinning, detachment, abnormal sarcomere patterning and muscle structure.

•Further research will determine if these kinase genes are important regulators of autophagy of Filamin in muscle cells.

•Some questions for future research: do knockdowns of these genes cause accumulation of autophagic markers, such as p62 and ATG8?

Acknowledgements and References

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1. Brooks D, Naeem F, Stetsiv M, et al. Drosophila NUAK functions with Starvin/BAG3 in autophagic protein turnover. *PLoS Genet*. 2020;16(4):e1008700. Published 2020 Apr 22. doi:10.1371/journal.pgen.1008700

2. Mirzoyan Z, Sollazzo M, Allocca M, Valenza AM, Grifoni D, Bellosta P. *Drosophila melanogaster*: A Model Organism to Study Cancer. *Front Genet*. 2019;10:51. Published 2019 Mar 1. doi:10.3389/fgene.2019.00051