Actomyosin fibers in the spermatheca are under tension

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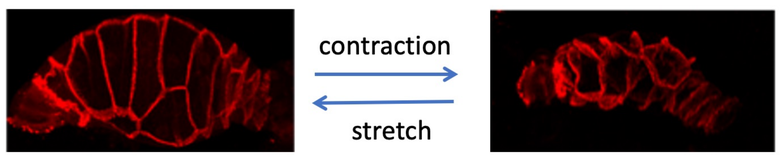
Regulating contractile and relaxation processes is crucial for the functionality of tubular structures in organisms. In *C. elegans*, the spermatheca, the site of fertilization, consists of smooth muscle-like cells. As oocytes pass through the spermatheca, they undergo multiple cycles of stretching and contracting. To understand the mechanical forces during ovulation, we employed the laser ablation tool and the strain sensor STReTCh to measure tension on the actin fibers within the spermatheca.

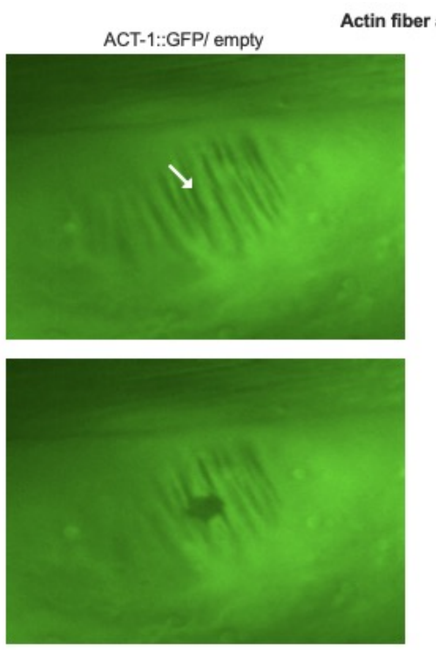
We utilized laser ablation to assess fiber tension. Using a SpectraPhysics Spirit laser, operating at 1040 nm, with 400 fs pulses at a frequency of 1 kHz and approximately 500 mW power, we severed the fibers in animals expressing ACT-1::GFP to monitor retraction rates.

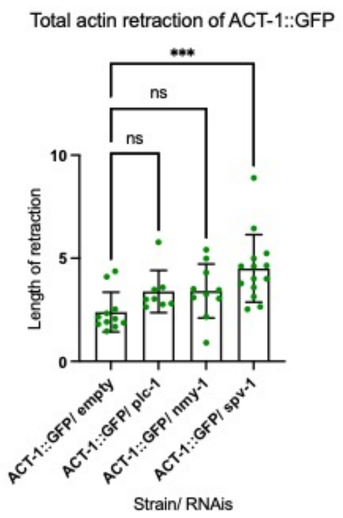
Additionally, the STReTCh sensor has been used to evaluate the tension on fibers. This system is composed of SpyTag and SpyCatcher, which interact when the mechanosensitive domain is unfolded. We adapted this method by integrating the sensor into mechanosensitive regions of DEB-1/vinculin and FLN-1/filamin in *C. elegans*. Our observations revealed that SpyCatcher paired with DEB-1/vinculin::SpyTag and FLN-1/filamin::SpyTag coincides in the spermatheca under *mel-11*/MYPT RNAi treatment and *plc-1*/phospholipase C RNAi treatment, respectively, indicating their role in fiber tension regulation.

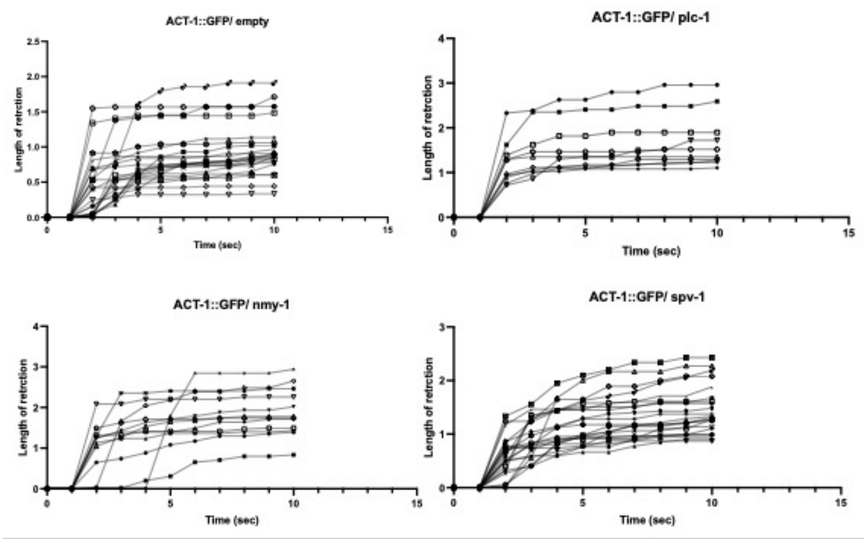
Our ongoing research aims to map tension distribution throughout the spermatheca and related proteins' regulatory role in contractility.



Laser severing releases tension on spermathecal actin fibers



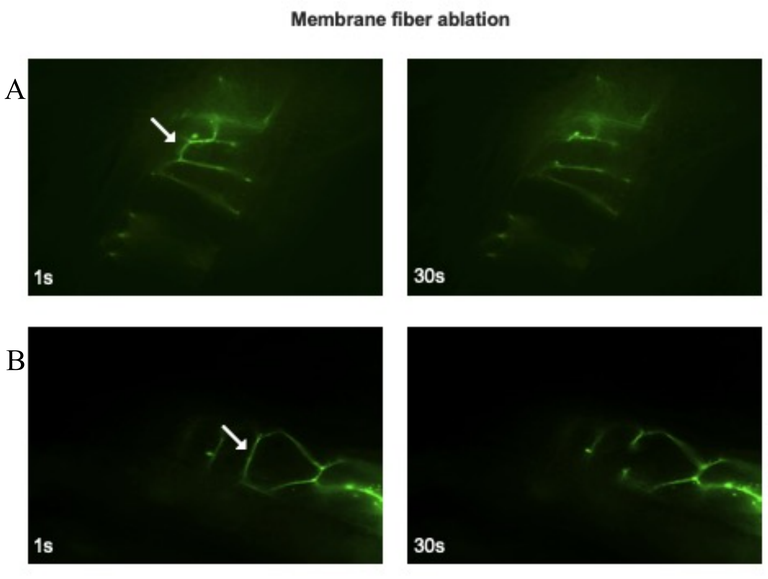




Laser severing of actin in ACT-1::GFP expressing animals treated with control, *plc-1, nmy-1,* and *spv-1* RNAi. Data indicates higher tension on *spv-1* RNAi treated animals. No significant differences between empty, *plc-1,*and *nmy-1* RNAi treatments.

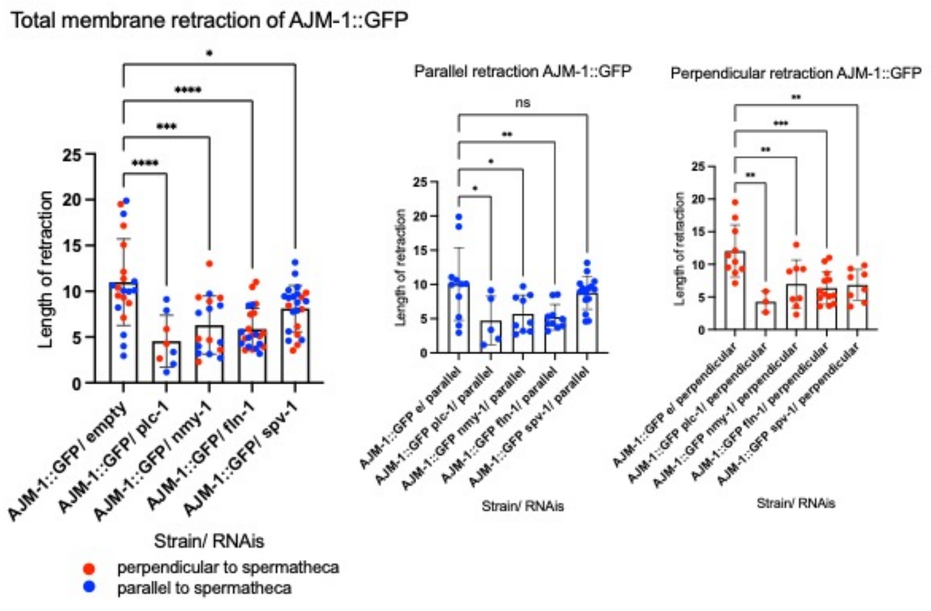
Analysis of length of retraction shows tension release of actin fibers after laser ablation of ACT-1::GFP expressing animals treated with control, *plc-1, nmy-1,* and *spv-1* RNAi.

Laser severing releases tension on spermathecal cell membrane



AJM-1::GFP expressing animals treated with control RNAi. Parallel (A) and perpendicular (B) spermathecal membranes severed using a SpectraPhysics Spirit 1040 nm laser, 400 fs pulses at 1kHz at ~500 μW power. Retraction indicates tension on cell membranes can be assessed.

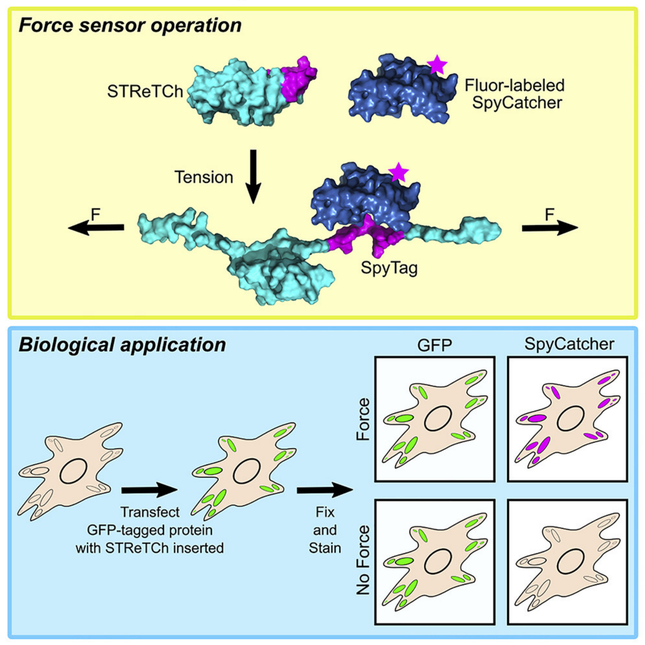
Total fiber retraction shows isotropic tension in spermatheca



Data indicates lower tension on membranes with *plc-1, nmy-1, fln-1,* and *spv-1* RNAi treatments compared to control in fibers perpendicular and parallel to the spermatheca.

Future Directions:

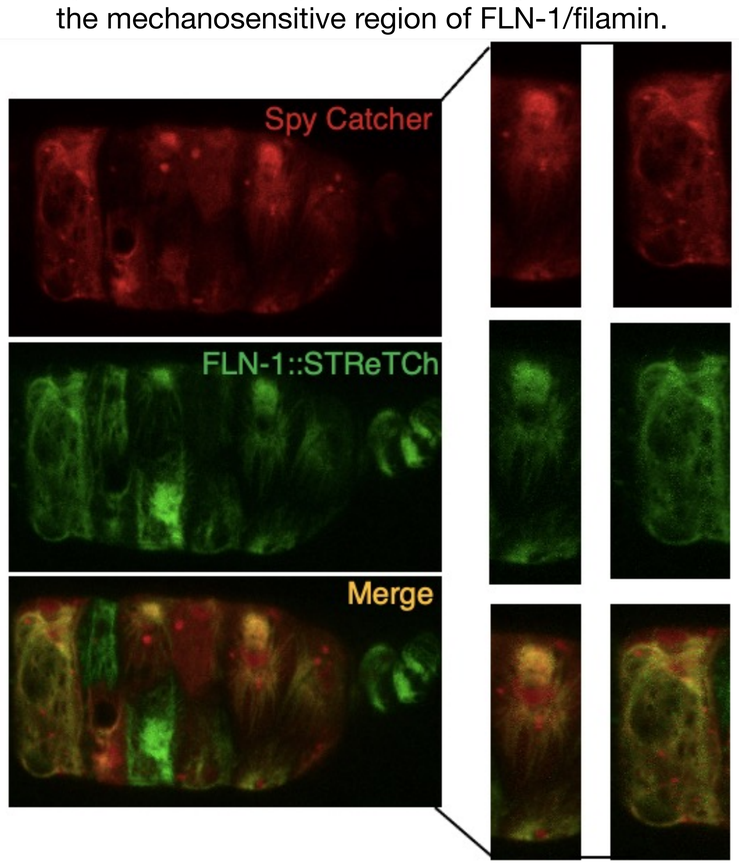
Measuring tension on fibers



Zhong et al,. 2022 Facile Detection of mechanical forces..with STReTCh

SpyCatcher and STReTCh module localization in FLN-1/filamin, DEB-1/vinculin, and HMR-1/cadherin

The Dunn lab inserted STReTCh in an internal vinculin site to assess tension. We adapted the system for *C. elegans* by inserting STReTCH in the mechanosensitive region of FLN-1/filamin.



1. SpyCatcher and FLN-1/filamin::STReTCh colocalize in the spermatheca when the animals treated with *plc-1*/phospholipase C RNAi.

2. SpyCatcher and DEB-1/vinculin::STReTCh colocalize in the spermatheca when the tissue contractility is increased with *mel-II*/ MYPT RNAi.

3. Next: insertion of STReTCh into HMR-1/Cadherin to study intracellular tension

Acknowledgements

This work is supported by a grant from the National Institutes of Health/National Institute of General Medical Sciences (GM110268) to E.J.C.
Thank you to CGC for strains.