Tension-sensitive recruitment of the RhoGEF RHGF-1 promotes actomyosin contractility in the *C. elegans* spermatheca

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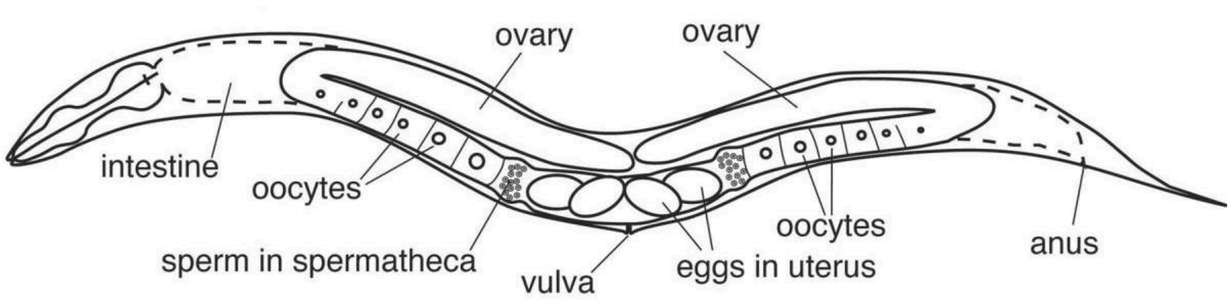
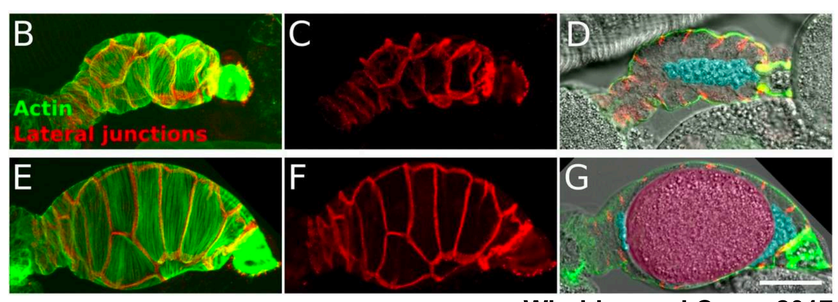
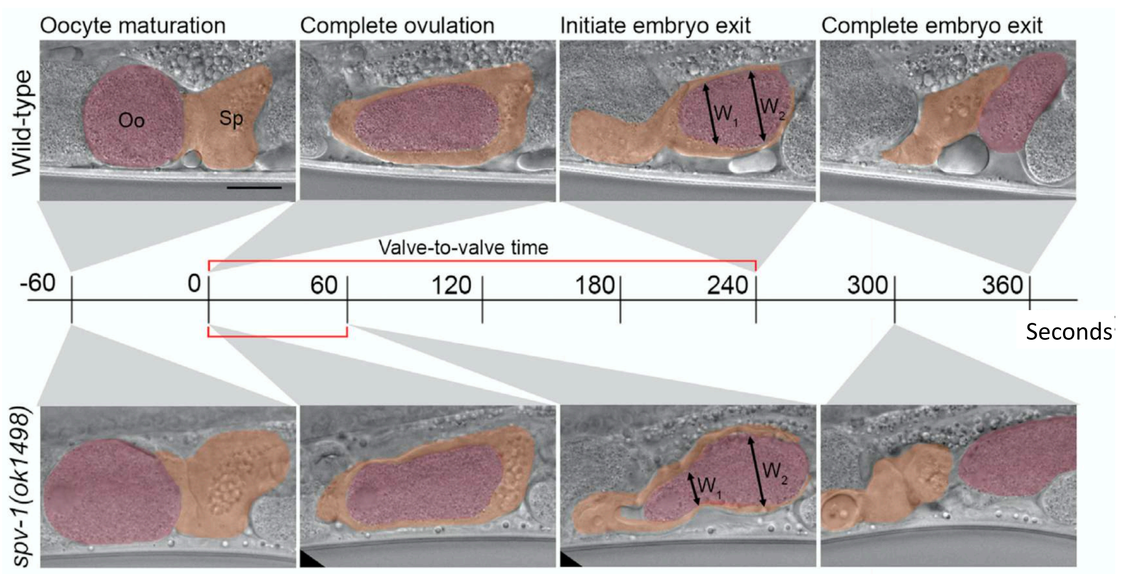
The *C. elegans* spermatheca, a flexible pouch like structure and a site for sperm storage, is an elegant model to study contractility at the tissue level. Each ovulation event involves the entry of a mature oocyte into the spermatheca, where it resides while it is fertilized and is then propelled forward into the uterus for further development. Oocyte entry dramatically stretches the myoepithelial cells that make up the spermatheca, and pushing out of the oocyte is the result of a wave of contractile forces generated by a circumferential actomyosin network within the spermatheca. Spermathecal contractility is regulated by calcium signaling and by the small GTPase protein, RHO-1.



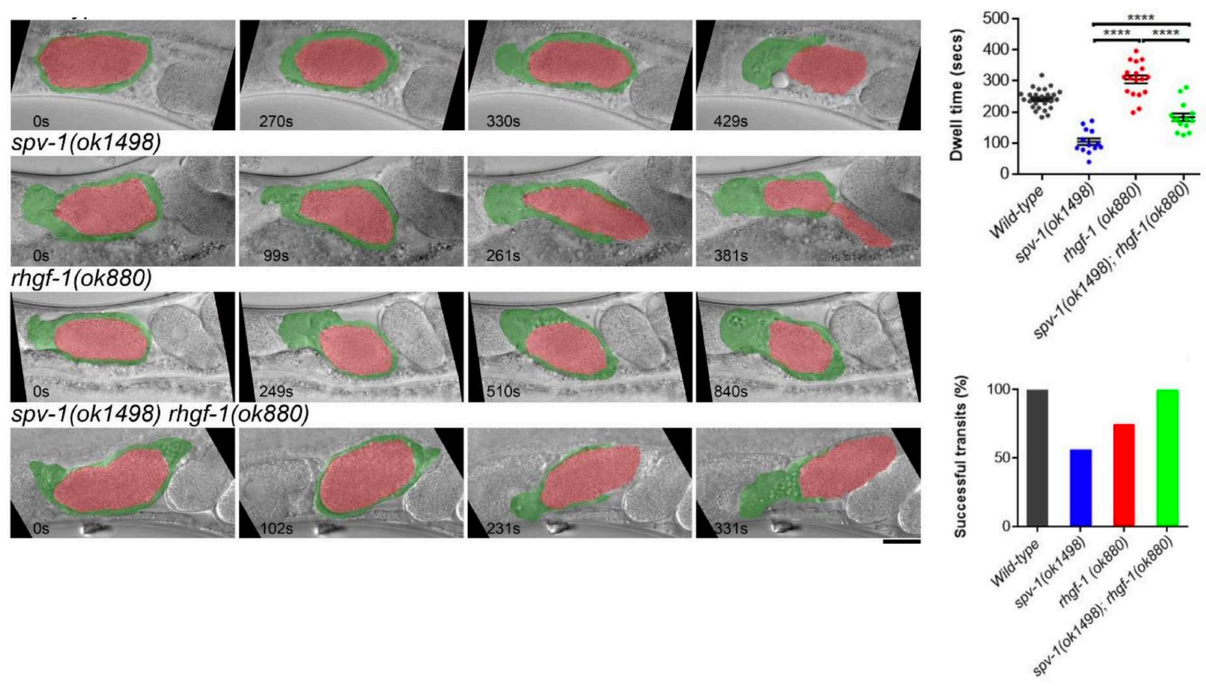


Wirshing and Cram, 2017

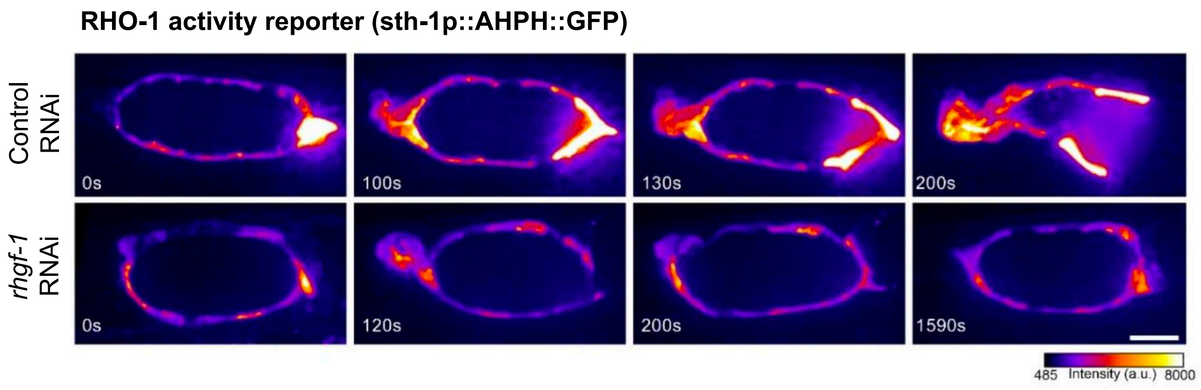
Previously, we identified a RhoGAP protein, SPV-1, which functions to inhibit premature RHO-1 activity and thus spermathecal contractility (Curr Biol. 2015 Jan 19;25(2):141-151.). However, the identity of the GEF/s that activate RHO-1 in the spermatheca was still unknown.

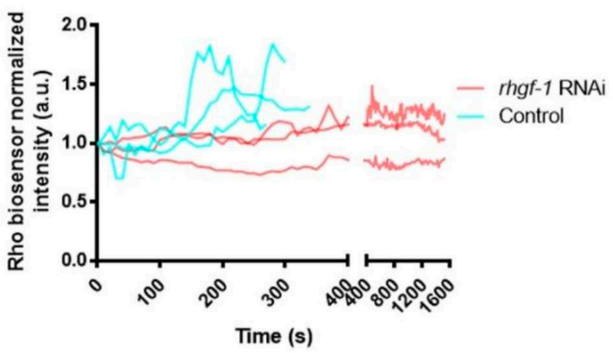


Here, we performed an RNAi screen for all the known *C. elegans* RhoGEFs in an *spv-1* loss-of-function mutant and identified RHGF-1 as a RhoGEF that rescues the hypercontractile phenotype of SPV-1 loss of function. On its own, RHGF-1 mutant has longer than normal transits and in a quarter of cases it traps the embryo in the spermatheca.

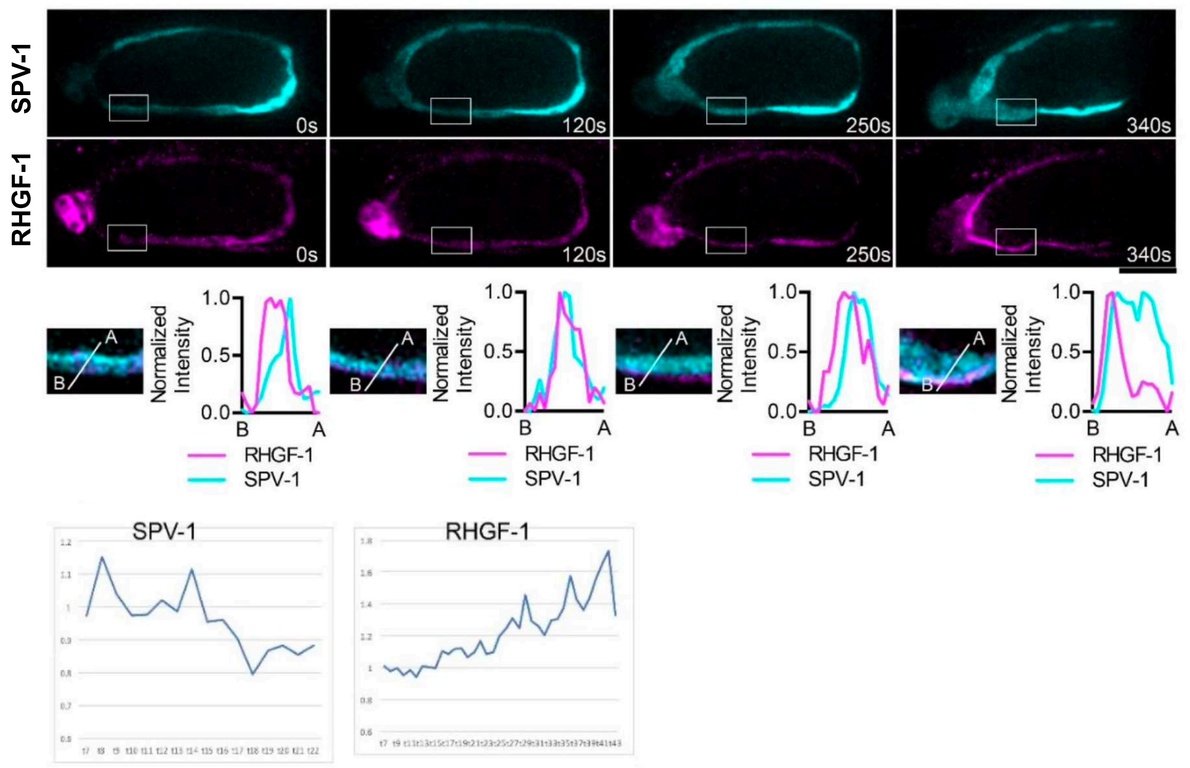


Using a RHO-1 biosensor, we found that upon depletion of RHGF-1, the level of active RHO-1 remains nearly constant and does not increase, as it does in WT, before spermatheca contraction

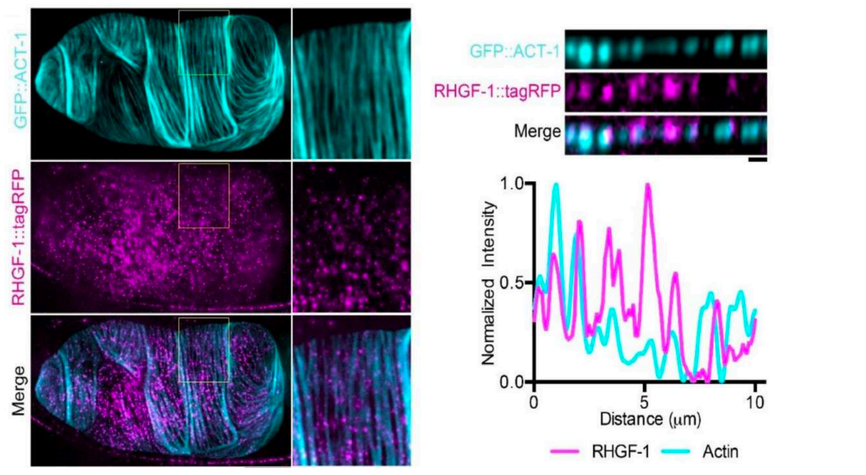


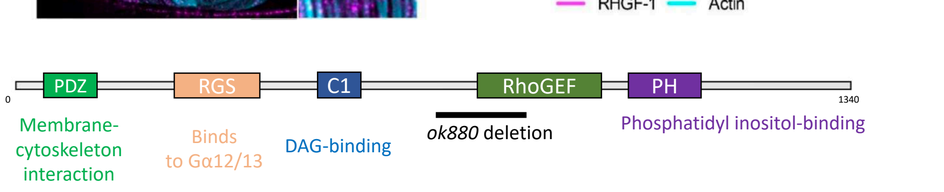


Using CRISPR-Cas9, we tagged RHGF-1 with tagRFP and live cell imaging showed RHGF-1 subcellular localization is dynamic and inverse to SPV-1: until oocyte entry it is cytoplasmic, but following stretching of the spermatheca, it is recruited to the basal side of the cell, eventually becoming concentrated there

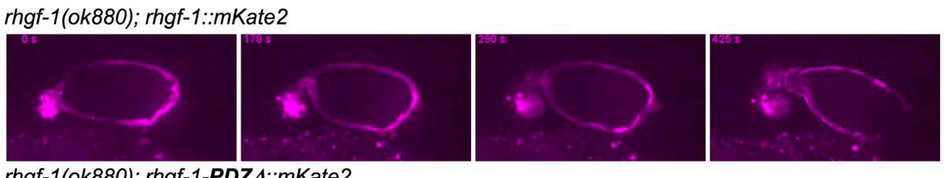


In live-super-resolution microscopy RHGF-1 appears to co-localize with F-actin bundles and structure/function domain deletion analysis reveals that its basal localization depends on an intact PDZ domain.

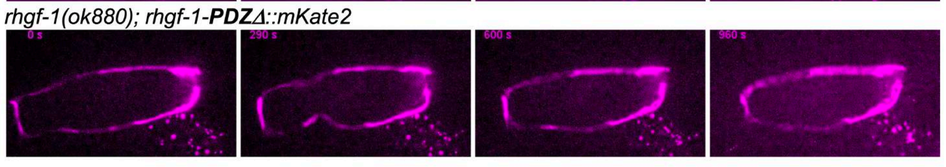




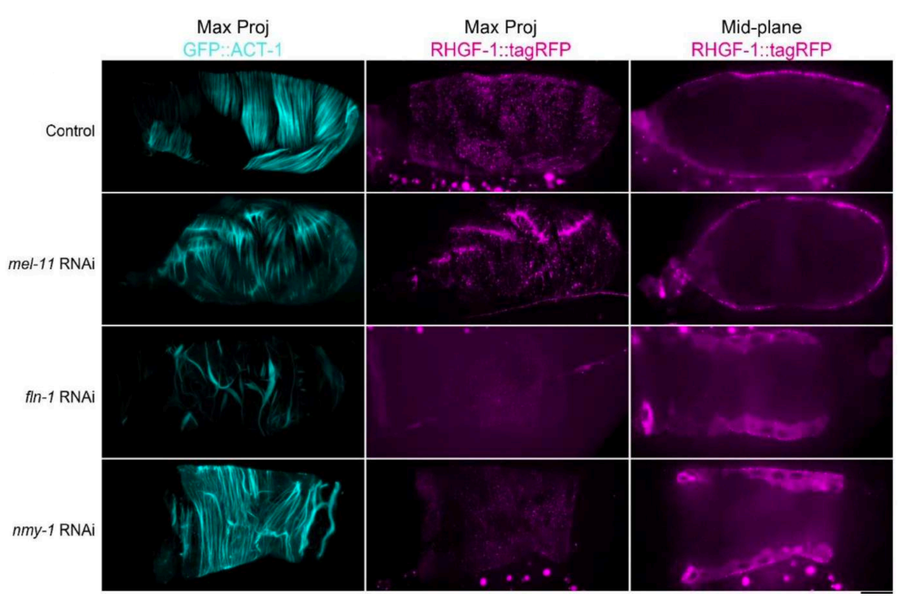
rhgf-1(ok880); rhgf-1::mKate2



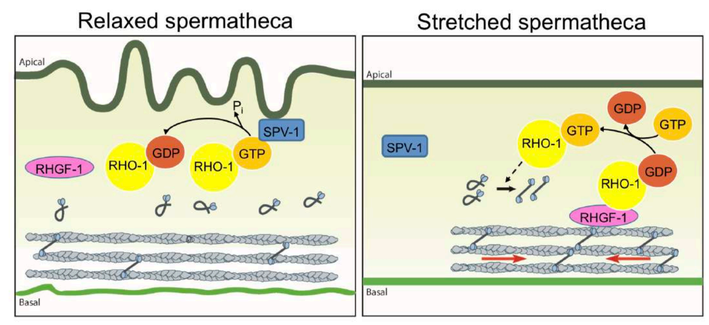
*rhgf-1(ok880); rhgf-1-**PDZ**Δ::mKate2*



Using genetic perturbations to vary the level of actomyosin contractility we found that RHGF-1 recruitment to F-actin bundles behaves in a tension-dependent manner.



In conclusion, RHGF-1 appears to function as a mechanosensor, detecting the stretch of the spermatheca upon oocyte entry and responding by relocating to F-actin and activating RHO-1 to induce spermatheca contraction.



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