# GPCRs, Contractility, and Calcium Signaling in the *C. elegans* Spermatheca

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## Abstract

The reproductive success of the worm *Caenorhabditis elegans (C. elegans)* largely depends on the ability of a mature oocyte to become fertilized in the spermatheca, the site for embryo fertilization. The spermatheca consists of the distal neck, the bag, and the spermatheca-uterine (sp-ut) valve. Immediately after oocyte entry, the sp-ut valve constricts. After ~10 minutes, the bag begins to constrict, and the valve relaxes allowing the embryo to exit the spermatheca and enter the uterus. These contractions are regulated by calcium signaling through PLC-1, which mediates calcium release from the ER. Here we study the role of G-protein coupled receptors (GPCR) in spermathecal contractility. We know that the GPCRs we are looking at play a role in the aforementioned contractions if their expression is knocked down and the contractions stop. This will result in the inability of the oocyte to exit the spermatheca. Single and double knockdown of the expression of the proteins Y40C5A.4 and NPR-35 were used to determine how depleted expression of these GPCRs affects the contraction of the spermatheca. We used Y40C3A.4(RNAi) and performed a trapping assay to determine if it was necessary to contraction in the spermatheca. We used *empty(RNAi)* and *plc-1(RNAi)* as our controls. Additionally, we depleted Y40C3A.4 in the strain VC2526, a null allele of *npr-35,* to determine if knocking down two GPCRs at the same time resulted in a phenotype. We used worms expressing GCaMP under a *fln-1* promoter to visualize calcium signaling in spermatheca in wild type worms and Y40C3A.4(RNAi) worms. In parallel, we are in the process of cloning RNAi vectors for AH9.4 and *npr-34*, two GPCRs expressed in the spermatheca. The project involved silencing genes with RNA interference (RNAi), molecular cloning, and fluorescence microscopy. There was a reduction of occupied spermatheca when we looked at Y40C3A.4(RNAi) compared to our *empty(RNAi)* control, and our Y40C3A.4(RNAi) movie obtained resulted in an short dwell time, but experiments need to be repeated. Y40C3A.4(RNAi) in an *npr-35* null background was comparable to our *empty(RNAi)* control.

## Background

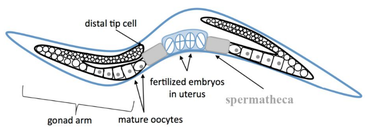



Fig. 1 Reproductive system of *C. elegans*

### The *C. elegans* spermatheca is a model of biological tubes

- Spermatheca: site of fertilization
  - Contractile tube
  - Stretched during ovulation
- Similarities between human smooth muscle systems
- Can be imaged while alive and intact
- ~150 ovulations per lifetime

### What is already known about the spermatheca?

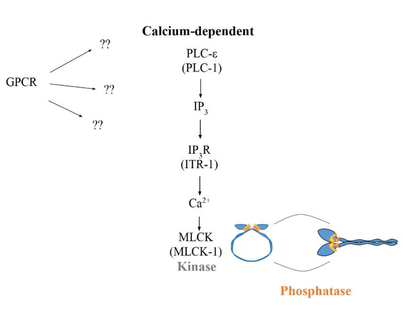



Fig. 2 Calcium signaling in the spermatheca

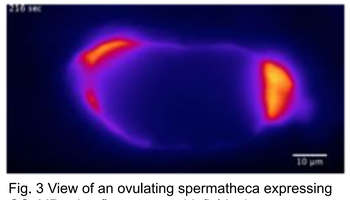
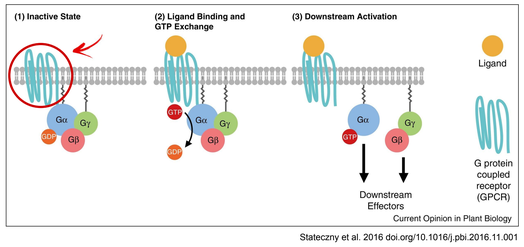


Fig. 3 View of an ovulating spermatheca expressing GCaMP using fluorescent widefield microscope

- Contractions are controlled by release of calcium
- In the presence of $Ca^{2+}$, GCaMP binds to the calcium, resulting in a conformational change and increased fluorescence

### What is the role of GPCRs in the contractility of the spermatheca?

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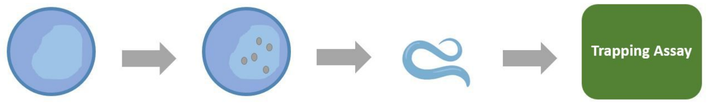
- G-protein coupled receptors regulate a multitude of different biological processes
- Activated by binding a ligand that transduce a signal through the cell via conformational changes

**The question we are addressing: How does the knockdown of specific GPCRs affect the contractility of the spermatheca?**

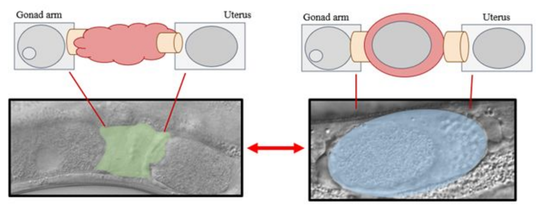
## Experimental Methods

### Knockdown of Gene via RNA Interference




- Bacteria obtained containing RNAi feeding vector. Cloning can be used to insert desired genes into RNAi vector L4440
- Bacteria seeded onto IPTG plates
- Worms are egg prepped and hatched into plates
- Worms ingest bacteria, the excess gained dsRNA causes the RNA to be degredated and therefore not expressed, essentially turning off the GPCR of interest
- Worms are prepared onto slides and observed under widefield microscope during a trapping assay. Occupied vs Unoccupied spermatheca are counted
- Trapping assay results allow us to determine if the relevant GPCR was significant enough to affect the normal contractile behavior in the spermatheca

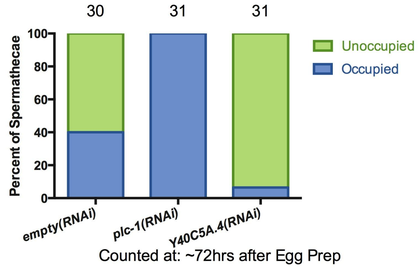
### Trapping Assay: Determining if GPCR plays a significant role in contractility of the spermatheca




- Wild type typically result in 20% occupied spermathecae at 24 hrs old
- If number of occupied spermatheca is considerably higher than 20%, GPCR may be responsible

Fig. 4 Comparing an unoccupied (green) vs. occupied (blue) spermatheca

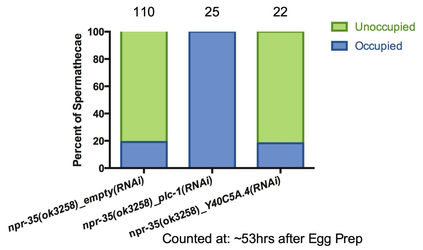
## Results




Counted at: ~72hrs after Egg Prep

Single Knockdown

- *empty(RNAi)* control yielded a 40% occupancy rate
- *plc-1(RNAi)* control yielded a 100% occupancy rate as expected
- Experimental gene Y40C5A.4 yielded only 6.5% occupancy (surprisingly lower than wild type trapping assays)

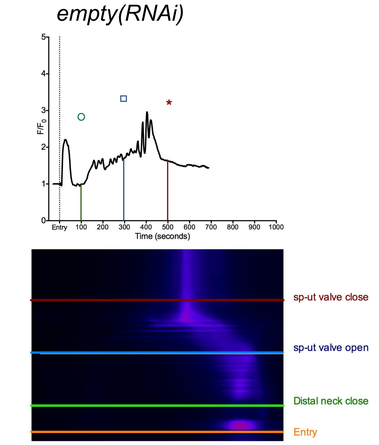


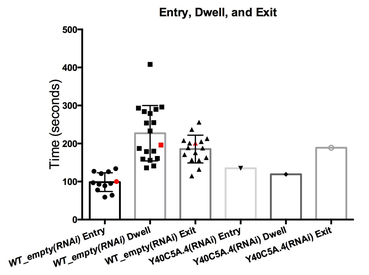

Counted at: ~53hrs after Egg Prep

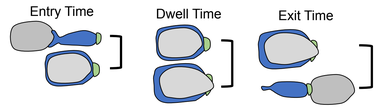
Double Knockdown

- *npr-35(ok3258)* has nonfunctional *npr-35*
- A previously known empty control gave an expected 20%
- The *plc-1(RNAi)* control gave us an expected 100%
- Experimental gene Y40C5A.4 yielded a 20% occupancy rate, putting it on par with the empty control

## Results Continued




- Kymograms give a spatial and temporal overview of the entirety of an ovulation based on calcium activity/levels
- Figures display entry, dwell, and exit time parameters measured

## Conclusion and Future Steps

Single Knockdown Gene Experiment

- The lack of expression of the Y40C5A.4 protein produced a significantly lower trapping assay percentage as compared to the negative control
- From this we conclude that the spermatheca of a worm certainly do not trap more due to the lack of expression of the gene. Therefore, the Y40C5A.4 gene could play a role in the contractions of that spermatheca, but further experimentation is needed.

Double Knockdown Gene Experiment

- Y540C5A.4 reduces spermathecal occupancy, but NPR-35 appears to partially reverse this effect, suggesting an interaction between these two genes. Two possible causes of reduced occupancy rate are more frequent contractions of the spermatheca, or the sp-ut valve being too loose. This idea is further supported by the shorter dwell time.

Further testing of other genes to find the ones that cause a higher percent trapping assay as well as confirming the results obtained, utilizing cloning to prepare bacteria with new RNA plasmids for AH9.4 and *npr-34*.

## Acknowledgements

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