Reverse genetic approaches for finding fertility genes in *C. elegans*

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INTRODUCTION

Fertilization is an essential process in all sexually reproducing species. Fertilization involves the fusion of an egg and a sperm cell to create an embryo. Species-specific sperm-egg interactions require complex protein-protein interactions on the sperm and egg surface, which are referred to as a fertilization synapse (Figure 1) (Krauchunas, et al.). While a lot of these genes that encode sperm surface proteins are identified, much less is known on the egg side of the equation.

The proteins of the *C. elegans* fertilization synapse

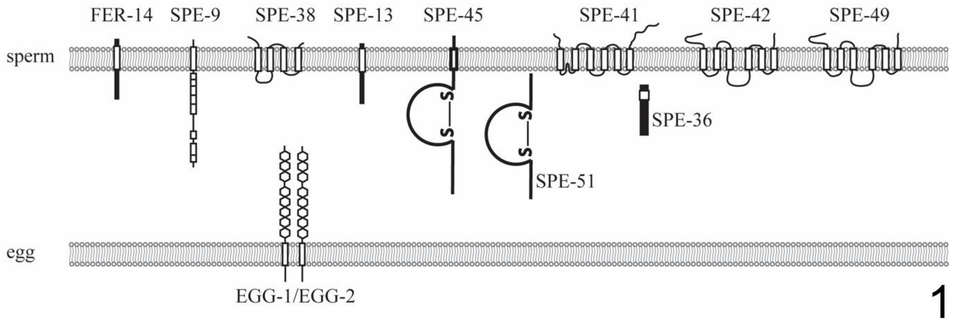

Figure 1. Above is a representation of some of the known proteins in a *C. elegans* fertilization synapse. We hope to discover more of the proteins on the *C. elegans* egg side of the synapse.

RESEARCH QUESTION OR OBJECTIVE

In order to discover some of these genes that encode potential egg-surface receptors for the sperm, I will be using a candidate genes approach to look for paralogous gene pairs that have potentially redundant functions.

METHODS

The process of refining data based on area of interest

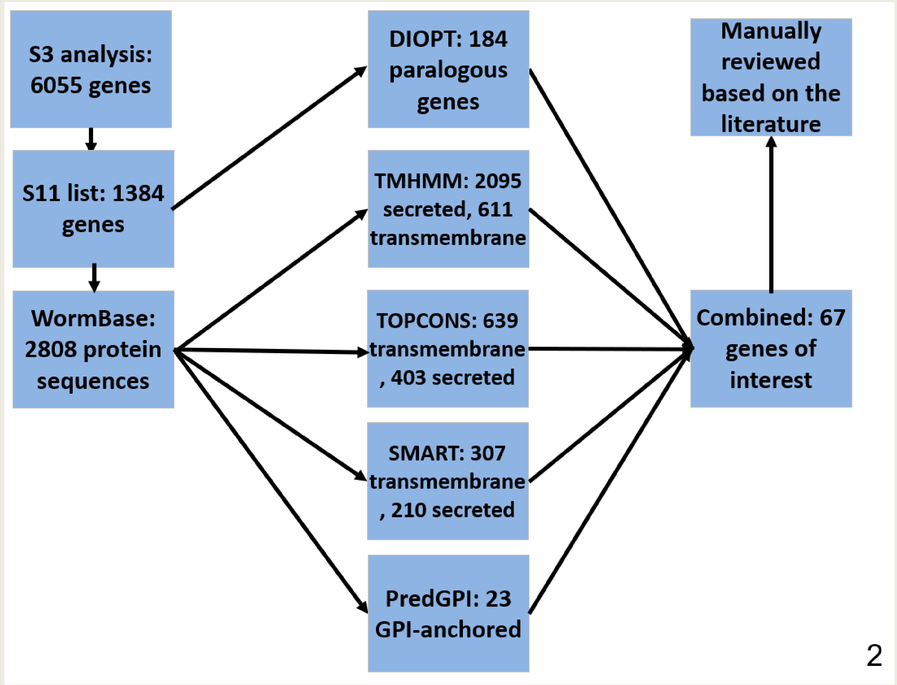

Figure 2. Each step of the data refinement discussed in this poster is demonstrated here as well as how each data set is used and changed in the process.

METHODS CONT.

- Starting dataset, Tzur's RNA-seq analysis: 6055 germline expressed genes (S3)
  - Filtering gene expression for oocyte-making germline generated: 1384 oogenesis-enriched gene list (S11)
- WormBase was used to find 2808 protein sequences for those 1384 genes (many genes encoded multiple proteins)
- Four domain predictors were then used:
  - TMHMM / TOPCONS / SMART for transmembrane, signal and secreted proteins
  - PredGPI for GPI-anchored proteins
- DIOPT was then used to filter the domain predictor master list for paralogous genes

RESULTS

The procedure discussed in methods left us with 67 genes to research. These genes were then broken up into 35 rows of paralogs. Finally, each gene was manually reviewed to determine its relevancy to fertilization.

The end result

emb-9	emb-9 encodes the alpha-1 chain of Type IV basement membrane collagen. It is expresed by body wall muscles and somatic gonad cells	**let-2**	let-2 encodes an alpha-2 type IV collagen. It is required for embryonic development and is part of the basement membrane between muscle		
let-23	let-23 encodes an EGF-receptor-family transmembrane tyrosine kinase. It is expressed in vulval precursor cells and the ALA neuron.	**cam-1**	cam-1 encodes a receptor tyrosine kinase of the immunoglobulin superfamily	**lin-18**	lin-18 encodes a predicted receptor tyrosine kinase that is a member of the Ryk/Derailed family of tyrosine kinase-related receptors
snt-4	Is an ortholog of human SYT4 (synaptotagmin 4). Is expressed in intestine.	**snt-5**	Is predicted to have several functions, including calcium ion binding activity; phospholipid binding activity; and syntaxin binding activity.		

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Figure 3.A few examples from the final list. Each row is a paralogous pair.

After being sorted into rows the gene pairs were further ranked by:
- Degree of expression in the germline
- Relevant mutant or knockdown phenotypes
- Their protein domain features
- Any relevant literature on phenotypes or other aspects of protein function

They were then color-coded green, yellow and red which quickly shows the most to least promising candidate genes

CONCLUSIONS

We have identified many candidate gene pairs that encode egg-surface receptors for the sperm.

We developed an efficient, python-based method to sort and refine the candidate gene list. In the future multiple additional datasets can be easily analyzed using this method.

For future work we will use gene knockdown or knockout in order to test the function of these genes during fertilization.

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